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DATE: Monday, June 14, 2004

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L12: Entry 1 of 8 File: USPT Nov 11, 2003

US-PAT-NO: 6645528
DOCUMENT-IDENTIFIER: US 6645528 B1
**** See image for Certificate of Correction ****

TITLE: Porous drug matrices and methods of manufacture thereof

DATE-ISSUED: November 11, 2003

US-CL-CURRENT: 424/489; 514/951

INT-CL: [07] A61 K 9/14

L12: Entry 2 of 8 File: USPT Aug 26, 2003

US-PAT-NO: 6610317
DOCUMENT-IDENTIFIER: US 6610317 B2

TITLE: Porous paclitaxel matrices and methods of manufacture thereof

DATE-ISSUED: August 26, 2003

US-CL-CURRENT: 424/422; 424/426, 424/489, 514/449

INT-CL: [07] A61 F 2/00, A61 F 9/14, A61 F 31/335

L12: Entry 3 of 8 File: USPT Jul 8, 2003

US-PAT-NO: 6589557
DOCUMENT-IDENTIFIER: US 6589557 B2

TITLE: Porous celecoxib matrices and methods of manufacture thereof

DATE-ISSUED: July 8, 2003

US-CL-CURRENT: 424/484; 424/400, 424/405, 424/489

INT-CL: [07] A61 K 9/14, A61 K 9/00

L12: Entry 4 of 8 File: USPT Aug 20, 2002

US-PAT-NO: 6437143
DOCUMENT-IDENTIFIER: US 6437143 B1

TITLE: Thiazolidone-2 derivatives, 4-diketone substituted, method for obtaining them and pharmaceutical compositions containing same

DATE-ISSUED: August 20, 2002

US-CL-CURRENT: 548/183

INT-CL: [07] C07 D 277/34, A61 K 31/426

L12: Entry 5 of 8 File: USPT Sep 4, 2001

US-PAT-NO: 6284269
DOCUMENT-IDENTIFIER: US 6284269 B1

TITLE: Pharmaceutical compositions of meloxicam with improved solubility and bioavailability

DATE-ISSUED: September 4, 2001

US-CL-CURRENT: 424/461; 424/462, 424/479, 424/493, 424/499

INT-CL: [07] A61 K 9/62

L12: Entry 6 of 8 File: USPT Mar 13, 2001

US-PAT-NO: 6200590
DOCUMENT-IDENTIFIER: US 6200590 B1

TITLE: Controlled, phased-release suppository and its method of production

DATE-ISSUED: March 13, 2001

US-CL-CURRENT: 424/433; 424/434, 424/DIG.15, 514/965

INT-CL: [07] A01 N 43/22

L12: Entry 7 of 8 File: USPT Oct 26, 1999

US-PAT-NO: 5972333
DOCUMENT-IDENTIFIER: US 5972333 A

TITLE: Synthetic mammalian sulphamidase and genetic sequences encoding same

DATE-ISSUED: October 26, 1999

US-CL-CURRENT: 424/94.6; 435/188, 435/195, 530/350, 536/23.2

INT-CL: [06] A61 K 38/47, C12 N 9/14, C07 H 21/04, C07 K 1/00

L12: Entry 8 of 8 File: USPT Aug 11, 1987

US-PAT-NO: 4686215
DOCUMENT-IDENTIFIER: US 4686215 A

TITLE: Pharmaceutical composition and method for treating tumors susceptible to 2-carbamoylaziridine

DATE-ISSUED: August 11, 1987

US-CL-CURRENT: 514/183

INT-CL: [04] A61K 31/33

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L5: Entry 1 of 6 File: USPT Sep 17, 2002

US-PAT-NO: 6451339
DOCUMENT-IDENTIFIER: US 6451339 B2
**** See image for Certificate of Correction ****

TITLE: Compositions and methods for improved delivery of hydrophobic agents

DATE-ISSUED: September 17, 2002

US-CL-CURRENT: 424/451; 424/435, 424/450, 424/455, 424/456, 424/463, 424/464, 424/489, 424/499, 424/502, 514/937, 514/938, 514/939, 514/940, 514/941, 514/942, 514/943, 514/975

INT-CL: [07] A61 K 9/127

L5: Entry 2 of 6 File: USPT May 7, 2002

US-PAT-NO: 6383471
DOCUMENT-IDENTIFIER: US 6383471 B1

TITLE: Compositions and methods for improved delivery of ionizable hydrophobic therapeutic agents

DATE-ISSUED: May 7, 2002

US-CL-CURRENT: 424/45; 424/401, 424/436, 424/451, 424/46, 514/944

INT-CL: [07] A61 K 9/12

L5: Entry 3 of 6 File: USPT Jan 22, 2002

US-PAT-NO: 6340479
DOCUMENT-IDENTIFIER: US 6340479 B1

TITLE: Stable, homogeneous, extract free or nearly free form secondary reaction products

DATE-ISSUED: January 22, 2002

US-CL-CURRENT: 424/725; 424/400, 424/730, 424/754, 424/756

INT-CL: [07] A61 K 35/78

L5: Entry 4 of 6 File: USPT Oct 30, 2001

US-PAT-NO: 6309663
DOCUMENT-IDENTIFIER: US 6309663 B1
**** See image for Certificate of Correction ****

TITLE: Triglyceride-free compositions and methods for enhanced absorption of hydrophilic therapeutic agents

DATE-ISSUED: October 30, 2001

US-CL-CURRENT: 424/450; 424/435, 424/451, 424/455, 424/456, 424/463, 424/464, 424/489, 424/499, 424/502, 514/937, 514/938, 514/939, 514/940, 514/941, 514/942, 514/943, 514/975

INT-CL: [07] A61 K 9/127

L5: Entry 5 of 6 File: USPT Jul 31, 2001

US-PAT-NO: 6267985
DOCUMENT-IDENTIFIER: US 6267985 B1

TITLE: Clear oil-containing pharmaceutical compositions

DATE-ISSUED: July 31, 2001

US-CL-CURRENT: 424/451; 424/43, 424/433, 424/436, 424/441, 424/443, 424/455, 424/456, 424/458, 424/463, 424/464, 424/465 , 424/489, 424/490, 424/731, 424/735, 424/750, 424/757, 424/764, 514/44, 514/772.2, 514/772.3, 514/777, 514/778, 514/779, 514/781, 514/783, 514/784, 514/785, 514/786, 514/937, 514/944

INT-CL: [07] A61 K 9/08, A61 K 9/10, A61 K 9/14, A61 K 9/20, A61 K 9/48

L5: Entry 6 of 6 File: USPT Dec 29, 1998

US-PAT-NO: 5853767
DOCUMENT-IDENTIFIER: US 5853767 A

TITLE: Compositions for treating fungal, parasitic and/or bacterial infections, especially infections of organs such as the skin and vagina

DATE-ISSUED: December 29, 1998

US-CL-CURRENT: 424/659; 514/557

INT-CL: [06] A61 K 33/22, A61 K 31/19

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☐ 1. Document ID: US 6645528 B1

L11: Entry 1 of 7 File: USPT Nov 11, 2003

DOCUMENT-IDENTIFIER: US 6645528 B1
** See image for Certificate of Correction **
TITLE: Porous drug matrices and methods of manufacture thereof

CLAIMS:

7. The composition of claim 1 wherein the matrix further comprise an excipient selected from the group consisting of hydrophilic polymers, sugars, tonicity agents, pegylated excipients, and combinations thereof.

11. The composition of claim 1 wherein the matrix is formed into suppositories suitable for vaginal or rectal administration.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw

☐ 2. Document ID: US 6610317 B2

L11: Entry 2 of 7 File: USPT Aug 26, 2003

DOCUMENT-IDENTIFIER: US 6610317 B2
TITLE: Porous paclitaxel matrices and methods of manufacture thereof

CLAIMS:

5. The composition of claim 1, wherein the matrix comprises at least one excipient selected from the group consisting of hydrophilic polymers, sugars, tonicity agents, pegylated excipients, and combination thereof.

9. The composition of claim 1 wherein the matrix is formed into suppositories suitable for vaginal or rectal administration.

14. The method of claim 11 wherein the taxane solution or pore forming agent comprises excipients selected from the group consisting of hydrophilic excipients, pegylated excipients, and tonicity agents.

25. The method of claim 18 wherein the formulation is in a suppository suitable for vaginal or rectal administration.

☐ 3. Document ID: US 6589557 B2

L11: Entry 3 of 7 File: USPT Jul 8, 2003

DOCUMENT-IDENTIFIER: US 6589557 B2
TITLE: Porous celecoxib matrices and methods of manufacture thereof

CLAIMS:

4. The method of claim 1 wherein the drug solution or pore forming agent further comprises an excipient selected from the group consisting of hydrophilic polymers, sugars, pegylated excipients, and tonicity agents.

11. The composition of claim 8 wherein the matrix further comprise an excipient selected from the group consisting of hydrophilic polymers, sugars, tonicity agents, pegylated excipients, and combinations thereof.

17. The composition of claim 8 wherein the matrix is formed into suppositories suitable for vaginal or rectal administration.

☐ 4. Document ID: US 6437143 B1

L11: Entry 4 of 7 File: USPT Aug 20, 2002

DOCUMENT-IDENTIFIER: US 6437143 B1
TITLE: Thiazolidone-2 derivatives, 4-diketone substituted, method for obtaining them and pharmaceutical compositions containing same

CLAIMS:

14. An injectable solution, suspension or multi-dose vial, a plain or coated tablet, a capsule, a pill, a cachet, a powder or a suppository comprising the pharmaceutical composition according to claim 11.

15. An excipient of a cellulose derivative, a microcrystalline cellulose, an alkaline-earth metal carbonate, a magnesium phosphate, a starch, a modified starch, a lactose, a cocoa butter, a polyethyleneglycol sterate, water, an aqueous solution,

solution, a physiological saline or a isotonic solution incorporating the pharmaceutical composition according to claim 11.

☐ 5. Document ID: US 6284269 B1

L11: Entry 5 of 7 File: USPT Sep 4, 2001

DOCUMENT-IDENTIFIER: US 6284269 B1
TITLE: Pharmaceutical compositions of meloxicam with improved solubility and bioavailability

CLAIMS:

10. Composition according to claim 1, characterized by methylcellulose-propylene-glycol ether, tris-hydroxymethylaminomethane, 2,6-diamino-hexanoic acid (D,L-lysine), mannitol, polyethyleneglycol, propyleneglycol, diethanolamine, ethyleneamine, monoethanolamine, triethanolamine, diisopropylamine, dibutylamine, pentylamine, sodium dodecylsulfate, methylglucamine, polyvinylpyrrolidone, cellulose ether, polyoxyethylene-polyoxypropylene-block-copolymers and/or nicotinamide as pharmaceutically acceptable additive.

16. Composition according to claim 11, characterized by i-propanol, propyleneglycol, glycerol, polyethyleneglycol and/or ethanol as co-solvent.

17. Composition according to claim 11, characterized by one ore more additional pharmaceutical acceptable additives selected from the group consisting of surfactants, hydrotrbpic agents, alkalizing agents, hydrocolloids and polymers, preferably selected from the group consisting of methylcellulose-propylene-glycol ether, tris-hydroxymethylaminomethane, 2,6-diamino-hexanoic acid (D,L-lysine), mannitol, polyethyleneglycol, propyleneglycol, diethanolamine, ethyleneamine, monoethanolamine, triethanolamine, diisopropylamine, dibutylamine, pentylamine, sodium carbonate, sodium dodecylsulfate, ammonium carbonate, sodium hydroxide, especially powdered sodium hydroxide, sodium phosphate, methylglucamine, polyvinylpyrrolidone, cellulose ether, polyoxyethylene-polyoxypropylene-block-copolymers and/or nicotinamide as pharmaceutically acceptable additive.

20. Composition according to claim 1, characterized in that it is provided as tablet, effervescent tablet, sachet, aromatized effervescent sachet, tab, hydrogel, ophthalmic ointment, ophthalmic hydrogel or retal suppository.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 6. Document ID: US 5972333 A

L11: Entry 6 of 7 File: USPT Oct 26, 1999

DOCUMENT-IDENTIFIER: US 5972333 A
TITLE: Synthetic mammalian sulphamidase and genetic sequences encoding same

CLAIMS:

15. The method according to any one of claims 10, 11, 12 or 13 wherein administration of the sulphamidase is by oral, intravenous, suppository, intraperitoneal, intramuscular, intranasal, intradermal or subcutaneous administration, by infusion, implantation or by gene therapy.

24. A chemically modified sulphamidase according to claim 23 wherein said sulphamidase has undergone interaction with polylysine or polyethyleneglycol.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw De

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☐ 7. Document ID: US 4686215 A

L11: Entry 7 of 7 File: USPT Aug 11, 1987

DOCUMENT-IDENTIFIER: US 4686215 A
TITLE: Pharmaceutical composition and method for treating tumors susceptible to 2-carbamoylaziridine

CLAIMS:

4. Method according to claim 1 wherein said administration is by suppository.

9. A pharmaceutical composition possessing antitumor activity against malignant tumors susceptible to 2-carbamoylaziridine and being in the form of tablets, dragees capsules, suppositories or ointments for administration to humans to effect said anti-malignant activity, said composition comprising an anti-malignant tumor effective amount of 2-carbamoylaziridine and a pharmaceutically acceptable filler.

10. The pharmaceutical composition of claim 9 wherein said pharmaceutical filler is selected from the group consisting of stearic acid, lactose, glucose, potato starch, talc, vegetable oils, and polyethyleneglycol.

12. The pharmaceutical composition of claim 9 in the form of suppositories and wherein the content of said 2-carbamoylaziridine composition is 1-50% by weight.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw De

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Search Results - Record(s) 1 through 6 of 6 returned.

☐ 1. Document ID: US 6451339 B2

L4: Entry 1 of 6 File: USPT Sep 17, 2002

DOCUMENT-IDENTIFIER: US 6451339 B2
**** See image for Certificate of Correction ****
TITLE: Compositions and methods for improved delivery of hydrophobic agents

Detailed Description Text (75):
Although formulations specifically suited to oral administration are presently preferred, the compositions of the present invention can also be formulated for topical, transdermal, ocular, pulmonary, vaginal, rectal, transmucosal or parenteral administration, in the form of a triglyceride-free cream, lotion, ointment, suppository, gel or the like. If such a formulation is desired, other additives may be included, such as are well-known in the art, to impart the desired consistency and other properties to the formulation. The compositions of the present invention can also be formulated as a spray or an aerosol. In particular, the compositions may be formulated as a sprayable solution, and such formulation is particularly useful for spraying to coat a multiparticulate carrier, such as a bead. Such multiparticulate carriers are well known in the art.

CLAIMS:

80. The formulation of claim 1, comprising a solution, a cream, a lotion, an ointment, a suppository, a spray, an aerosol, a paste or a gel.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw. De

☐ 2. Document ID: US 6383471 B1

L4: Entry 2 of 6 File: USPT May 7, 2002

DOCUMENT-IDENTIFIER: US 6383471 B1
TITLE: Compositions and methods for improved delivery of ionizable hydrophobic therapeutic agents

Brief Summary Text (152):
Although formulations specifically suited to oral administration are presently

preferred, the compositions of the present invention can also be formulated for topical, transdermal, ocular, pulmonary, vaginal, rectal, transmucosal or parenteral parenteral administration, in the form of a cream, lotion, ointment, suppository, gel or the like. If such a formulation is desired, other additives may be included, such as are well-known in the art, to impart the desired consistency and other properties to the formulation. The compositions of the present invention can also be formulated as a spray or an aerosol. In particular, the compositions may be formulated as a sprayable solution, and such formulation is particularly useful for spraying to coat a multiparticulate carrier, such as a bead. Such multiparticulate carriers are well known in the art.

CLAIMS:

70. A dosage form comprising the pharmaceutical composition of claim 1 formulated as a solution, a cream, a lotion, an ointment, a suppository, a spray, an aerosol, a paste or a gel.

107. The method of claim 105, wherein the dosage form is selected from the group consisting of a capsule, a solution, a cream, a lotion, an ointment, a suppository, a spray, an aerosol, a paste and a gel.

☐ 3. Document ID: US 6340479 B1

L4: Entry 3 of 6 File: USPT Jan 22, 2002

DOCUMENT-IDENTIFIER: US 6340479 B1
TITLE: Stable, homogeneous, extract free or nearly free form secondary reaction products

CLAIMS:

31. The extract according to claim 28, wherein said semisolid administrative form is selected from the group consisting of a cream, a gel, an ointment, a paste and a suppository.

☐ 4. Document ID: US 6309663 B1

L4: Entry 4 of 6 File: USPT Oct 30, 2001

DOCUMENT-IDENTIFIER: US 6309663 B1
**** See image for Certificate of Correction ****
TITLE: Triglyceride-free compositions and methods for enhanced absorption of

hydrophilic therapeutic agents

Brief Summary Text (208):
Although formulations specifically suited to oral administration are presently preferred, the compositions of the present invention can also be formulated for topical, transdermal, buccal, nasal, ocular, pulmonary, vaginal, rectal, transmucosal or parenteral administration, as well as for oral administration. Thus, the dosage form can be a solution, suspension, emulsion, cream, ointment, lotion, suppository, spray, aerosol, paste, gel, drops, douche, ovule, wafer, troche, cachet, syrup, elixer, or other dosage form, as desired. If formulated as a suspension, the composition can further be processed in capsule form.

CLAIMS:

64. The pharmaceutical system of claim 1, wherein the dosage form of the composition is a solution, suspension, emulsion, cream, ointment, lotion, suppository, spray, aerosol, paste, gel, drops, douche, ovule, wafer, troche, cachet, syrup or elixir.

141. The pharmaceutical system of claim 76, wherein the dosage form of the composition is a solution, suspension, emulsion, cream, ointment, lotion, suppository, spray, aerosol, paste, gel, drops, douche, ovule, wafer, troche, cachet, syrup or elixir.

☐ 5. Document ID: US 6267985 B1

L4: Entry 5 of 6 File: USPT Jul 31, 2001

DOCUMENT-IDENTIFIER: US 6267985 B1
TITLE: Clear oil-containing pharmaceutical compositions

Brief Summary Text (163):
Although formulations specifically suited to oral administration are presently preferred, the compositions of the present invention can also be formulated for topical, transdermal, buccal, ocular, pulmonary, vaginal, rectal, transmucosal or parenteral administration, as well as for oral administration. Thus, the dosage form can be a solution, suspension, emulsion, cream, ointment, lotion, suppository, spray, aerosol, paste, gel, drops, douche, ovule, wafer, troche, cachet, syrup, elixer, or other dosage form, as desired. If formulated as a suspension, the composition can further be processed in capsule form.

CLAIMS:

67. A dosage form comprising the pharmaceutical composition of claim 1, wherein the dosage form is selected from the group consisting of a solution, suspension, emulsion, cream, ointment, lotion, suppository, spray, aerosol, paste, gel, drops, douche, ovule, wafer, troche, cachet, syrup and elixir.

135. A dosage form comprising the pharmaceutical composition of claim 75, wherein the dosage form is selected from the group consisting of a solution, suspension,

emulsion, cream, ointment, lotion, suppository, spray, aerosol, paste, gel, drops, douche, ovule, wafer, troche, cachet, syrup and elixir.

155. The method of claim 151, wherein the dosage form is selected from the group consisting of a solution, suspension, emulsion, cream, ointment, lotion, suppository, spray, aerosol, paste, gel, drops, douche, ovule, wafer, troche, cachet, syrup and elixir.

☐ 6. Document ID: US 5853767 A

L4: Entry 6 of 6 File: USPT Dec 29, 1998

DOCUMENT-IDENTIFIER: US 5853767 A
TITLE: Compositions for treating fungal, parasitic and/or bacterial infections, especially infections of organs such as the skin and vagina

Brief Summary Text (10):
Acetic acid is known to have antimicrobial capacities and is effective at various percentages against Pseudomonas, Staphylococcus, Streptococcus and various yeasts. The fungitoxic effects of acetic acid on fungi causing otomycosis has been examined in Mycoses 37: 299-301 (1994). In this study various volatile compounds, including acetic acid, were tested, in vitro, for their fungitoxic effects against five fungi. Some antifungal effect was illustrated for acetic acid. Candida albicans was shown to be the most resistant to the volatiles. Boric acid is known to be an effective antibacterial and antifungal agent and has been prepared in the form of a suppository for the treatment of fungal infection alone (see The Annals of Pharmacotherapy 1993, Volume 27, pp.1355-1357). Boric acid has been shown to be fungistatic against Candida albicans wherein in vitro tests show that it is effective in treating vulvovaginal candidiasis (see Obstet. Gynecol., (1974) 43:893-895, "Boric Acid Treatment of Vulvovaginitis").

Brief Summary Text (19):
Such a composition is most preferably administered in the form of a suppository although other dosage forms are also advantageously envisioned. Advantages to administering the composition as a suppository include convenience, ease of application, increased safety and neatness. Other dosage forms include solutions, for douching and the like, creams, ointments, gels, creme rinses and foams.

Brief Summary Text (27):
The composition of the present invention is in any form suitable for treatment of the vagina and skin, such as, for example, solutions, gels, shampoos, creams, creme rinses, ointments, suppositories, tablets and powders. The amount of acetic acid and and boric acid in the composition is an amount which is safe and effective and varies depending on the nature of the composition, the organ and animal being treated and the severity of the infection. Such an amount is determinable by a person of skill in the art. Acetic acid preferably comprises about 0.1% to 10.0% by weight of the composition, more preferably 2.0-5.0% by weight of the composition. Boric acid preferably comprises about 0.1% to 30.0% by weight of the composition, more preferably about 2.0-12.0% by weight of the composition.

Brief Summary Text (28):

Where the composition is applied as a suppository, the active ingredients are combined with inert suppository bases, depending on the nature of the suppository, such as cocoa butter, glycerinated gelatin, hydrogenated vegetables oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol. Means of creating these suppository bases are known to those skilled in the art. The use of soluble or dispersible bases such as polyethylene glycols or glycol surfactant combinations has the substantial advantage of lack of dependence on melting point approximating body temperature. Moreover, handling, storage and shipping are considerably simplified.

Brief Summary Text (29):
The preparation of such suppository compositions includes well known techniques of rolling (hand shaping), molding (fusion) and cold compression. Suppositories are usually globular or oviform and weigh about 5 gram. Reference is made to Remington's Pharmaceutical Sciences, 18th Edition, Chapter 87, pages 1609-13 (1990), the disclosure of which is expressly incorporated herein by reference.

Brief Summary Text (37):
As will be understood by those skilled in the art, the regimen for treating vaginal infection and/or skin conditions will depend on the severity of the infection and the form of the composition. By way of example, where the composition is in the form of a cream, the cream is topically applied to the affected area. Where the composition is in the form of a suppository, the suppository is inserted into the vagina, most preferably twice daily for 7 days.

Detailed Description Text (2):
EXAMPLE 1--VAGINAL SUPPOSITORY

Detailed Description Text (3):
A suitable formulation for a composition in the form of a suppository for treating vaginal infection is given as follows:

Detailed Description Text (8):
The suppository essentially comprising the above formulation can be prepared as mentioned above in accordance with well known techniques in the art. The amount of cocoa butter may vary but will be sufficient to compound the suppository.

Detailed Description Text (10):
Treatment of the vaginal infection may very depending on the severity of the infection. In general, a suitable treatment regime would be to insert the suppository into the vagina, twice daily for 7 days.

Other Reference Publication (4):
Asikoglu et al., The Release of Isoconazole Nitrate From Different Suppository Bases: In-vitro Dissolution, Physicochemical and Microbiological Studies, (1995) (Asikoglu-1). Hart, Boric Acid Vaginal Suppositories, 27 Annals of Pharmacotherapy 1355 (1993) (Hart).

Other Reference Publication (5):
Hart, Boric Acid Vaginal Suppositories, 27 Annals of Pharmacotherapy 1355 (1993) (Hart).

CLAIMS:

1. A composition comprising acetic acid in an amount between 0.1-10% by weight of the total composition and boric acid in an amount between 0.1-30% by weight of the total composition; wherein said composition is in a form suitable for vaginal treatment being selected from the group consisting of vaginal suppository, vaginal douche, vaginal shampoo, vaginal cream, vaginal ointment; vaginal gel, vaginal creme rinse, vaginal foam and vaginal solution.

2. The composition of claim 1 wherein the composition is in the form of a vaginal suppository.

10. A composition in the form of a vaginal suppository comprising 600 mg boric acid, 0.1-10% by weight glacial acetic acid, wherein said percentages are by weight of the total composition.

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☐ 1. Document ID: US 6730293 B1

L3: Entry 1 of 13 File: USPT May 4, 2004

DOCUMENT-IDENTIFIER: US 6730293 B1
TITLE: Compositions and methods for treating inflammatory diseases of the skin

Detailed Description Text (14):
The terms "non-polypeptide agent" and "non-polypeptide therapeutic agent" refer to the portion of a conjugate that does not include the delivery-enhancing transporter, and that is a biologically active agent other than a polypeptide. An example of a non-polypeptide agent is an anti-sense oligonucleotide, which can be conjugated to a poly-arginine peptide to form a conjugate for enhanced delivery into and across one or more layers of an epithelial or endothelial tissue.

Detailed Description Text (24):
The delivery-enhancing transporters increase delivery of the conjugate into and across one or more intact epithelial or endothelial tissue layers compared to delivery of the compound in the absence of the delivery-enhancing transporter. The delivery-enhancing transporters can, in some embodiments, increase delivery of the conjugate significantly over that obtained using the tat protein of HIV-1 (Frankel et al. (1991) PCT Pub. No. WO 91/09958). Delivery is also increased significantly over the use of shorter fragments of the tat protein containing the tat basic region (residues 49-57 having the sequence RKKRRQRRR) (Barsoum et al. (1994) WO 94/04686 and Fawell et al. (1994) Proc. Nat'l. Acad. Sci. USA 91: 664-668). Preferably, delivery obtained using the transporters of the invention is increased more than 2-fold, still more preferably six-fold, still more preferably ten-fold, and still more preferably twenty-fold, over that obtained with tat residues 49-57.

Detailed Description Text (119):
The active compounds of the formulas may be formulated into a suppository comprising, for example, about 0.5% to about 50% of a compound of the invention, disposed in a polyethylene glycol (PEG) carrier (e.g., PEG 1000 [96%] and PEG 4000 [4%]).

Detailed Description Text (167):
The enhanced transport methods of the invention are particularly suited for enhancing transport into and across one or more layers of an epithelial or endothelial tissue for a number of macromolecules, including, but not limited to proteins, nucleic acids, polysaccharides, and analogs thereof. Exemplary nucleic acids include oligonucleotides and polynucleotides formed of DNA and RNA, and analogs thereof, which have selected sequences designed for hybridization to complementary targets (e.g., antisense sequences for single- or double-stranded targets), or for expressing nucleic acid transcripts or proteins encoded by the sequences. Analogs include charged and preferably uncharged backbone analogs, such

as phosphonates (preferably methyl phosphonates), phosphoramidates (N3' or N5'), thiophosphates, uncharged morpholino-based polymers, and protein nucleic acids (PNAs). Such molecules can be used in a variety of therapeutic regimens, including enzyme replacement therapy, gene therapy, and anti-sense therapy, for example.

Detailed Description Text (168):
By way of example, protein nucleic acids (PNA) are analogs of DNA in which the backbone is structurally homomorphous with a deoxyribose backbone. The backbone consists of N-(2-aminoethyl)glycine units to which the nucleobases are attached. PNAs containing all four natural nucleobases hybridize to complementary oligonucleotides obeying Watson-Crick base-pairing rules, and is a true DNA mimic in terms of base pair recognition (Egholm et al. (1993) Nature 365:566-568. The backbone of a PNA is formed by peptide bonds rather than phosphate esters, making it well-suited for anti-sense applications. Since the backbone is uncharged, PNA/DNA PNA/DNA or PNA/RNA duplexes that form exhibit greater than normal thermal stability. PNAs have the additional advantage that they are not recognized by nucleases or proteases. In addition, PNAs can be synthesized on an automated peptides synthesizer using standard t-Boc chemistry. The PNA is then readily linked to a transport polymer of the invention.

Detailed Description Text (169):
Examples of anti-sense oligonucleotides whose transport into and across epithelial and endothelial tissues can be enhanced using the methods of the invention are described, for example, in U.S. Pat. No. 5,594,122. Such oligonucleotides are targeted to treat human immunodeficiency virus (HIV). Conjugation of a transport polymer to an anti-sense oligonucleotide can be effected, for example, by forming an an amide linkage between the peptide and the 5'-terminus of the oligonucleotide through a succinate linker, according to well-established methods. The use of PNA conjugates is further illustrated in Example 11 of PCT Application PCTJUS98/10571. FIG. 7 of that application shows results obtained with a conjugate of the invention containing a PNA sequence for inhibiting secretion of gamma-interferon (.gamma.-IFN) by T cells, as detailed in Example 11. As can be seen, the anti-sense PNA conjugate was effective to block .gamma.-IFN secretion when the conjugate was present at levels above about 10 .mu.M. In contrast, no inhibition was seen with the sense-PNA conjugate or the non-conjugated antisense PNA alone.

Detailed Description Text (172):
In another embodiment, the invention is useful for delivering immunospecific antibodies or antibody fragments to the cytosol to interfere with deleterious biological processes such as microbial infection. Recent experiments have shown that intracellular antibodies can be effective antiviral agents in plant and mammalian cells (e.g., Tavladoraki et al. (1993) Nature 366:469; and Shaheen et al. (1996) J Virol. 70:3392. These methods have typically used single-chain variable region fragments (scFv), in which the antibody heavy and light chains are synthesized as a single polypeptide. The variable heavy and light chains are usually separated by a flexible linker peptide (e.g., of 15 amino acids) to yield a 28 kDa molecule that retains the high affinity ligand binding site. The principal obstacle to wide application of this technology has been efficiency of uptake into infected cells. But by attaching transport polymers to scFv fragments, the degree of cellular uptake can be increased, allowing the immunospecific fragments to bind and disable important microbial components, such as HIV Rev, HIV reverse transcriptase, and integrase proteins.

☐ 2. Document ID: US 6669951 B2

L3: Entry 2 of 13 File: USPT Dec 30, 2003

DOCUMENT-IDENTIFIER: US 6669951 B2
TITLE: Compositions and methods for enhancing drug delivery across and into epithelial tissues

Detailed Description Text (14):
The terms "non-polypeptide agent" and "non-polypeptide therapeutic agent" refer to the portion of a conjugate that does not include the delivery-enhancing transporter, and that is a biologically active agent other than a polypeptide. An example of a non-polypeptide agent is an anti-sense oligonucleotide, which can be conjugated to a poly-arginine peptide to form a conjugate for enhanced delivery into and across one or more layers of an epithelial or endothelial tissue.

Detailed Description Text (23):
The delivery-enhancing transporters increase delivery of the conjugate into and across one or more intact epithelial or endothelial tissue layers compared to delivery of the compound in the absence of the delivery-enhancing transporter. The delivery-enhancing transporters can, in some embodiments, increase delivery of the conjugate significantly over that obtained using the tat protein of HIV-1 (Frankel et al. (1991) PCT Pub. No. WO 91/09958). Delivery is also increased significantly over the use of shorter fragments of the tat protein containing the tat basic region (residues 49-57 having the sequence RKKKRRQRRR; SEQ ID NO:28) (Barsoum et al. (1994) WO 94/04686 and Fawell et al. (1994) Proc. Nat'l. Acad. Sci. USA 91: 664-668). Preferably, delivery obtained using the transporters of the invention is increased more than 2-fold, still more preferably six-fold, still more preferably ten-fold, and still more preferably twenty-fold, over that obtained with tat residues 49-57.

Detailed Description Text (115):
The active compounds of the formulas may be formulated into a suppository comprising, for example, about 0.5% to about 50% of a compound of the invention, disposed in a polyethylene glycol (PEG) carrier (e.g., PEG 1000 [96%] and PEG 4000 [4%]).

Detailed Description Text (175):
The enhanced transport methods of the invention are particularly suited for enhancing transport into and across one or more layers of an epithelial or endothelial tissue for a number of macromolecules, including, but not limited to proteins, nucleic acids, polysaccharides, and analogs thereof. Exemplary nucleic acids include oligonucleotides and polynucleotides formed of DNA and RNA, and analogs thereof, which have selected sequences designed for hybridization to complementary targets (e.g., antisense sequences for single- or double-stranded targets), or for expressing nucleic acid transcripts or proteins encoded by the sequences. Analogs include charged and preferably uncharged backbone analogs, such as phosphonates (preferably methyl phosphonates), phosphoramidates (N3' or N5'), thiophosphates, uncharged morpholino-based polymers, and protein nucleic acids (PNAs). Such molecules can be used in a variety of therapeutic regimens, including enzyme replacement therapy, gene therapy, and anti-sense therapy, for example.

Detailed Description Text (176):
By way of example, protein nucleic acids (PNA) are analogs of DNA in which the backbone is structurally homomorphous with a deoxyribose backbone. The backbone consists of N-(2-aminoethyl)glycine units to which the nucleobases are attached. PNAs containing all four natural nucleobases hybridize to complementary oligonucleotides obeying Watson-Crick base-pairing rules, and is a true DNA mimic in terms of base pair recognition (Egholm et al. (1993) Nature 365:566-568. The

backbone of a PNA is formed by peptide bonds rather than phosphate esters, making it it well-suited for anti-sense applications. Since the backbone is uncharged, PNA/DNA PNA/DNA or PNA/RNA duplexes that form exhibit greater than normal thermal stability. PNAs have the additional advantage that they are not recognized by nucleases or proteases. In addition, PNAs can be synthesized on an automated peptides synthesizer using standard t-Boc chemistry. The PNA is then readily linked to a transport polymer of the invention.

Detailed Description Text (177):
Examples of anti-sense oligonucleotides whose transport into and across epithelial and endothelial tissues can be enhanced using the methods of the invention are described, for example, in U.S. Pat. No. 5,594,122. Such oligonucleotides are targeted to treat human immunodeficiency virus (HIV). Conjugation of a transport polymer to an anti-sense oligonucleotide can be effected, for example, by forming an an amide linkage between the peptide and the 5'-terminus of the oligonucleotide through a succinate linker, according to well-established methods. The use of PNA conjugates is further illustrated in Example 11 of PCT Application PCT/US98/10571. FIG. 7 of that application shows results obtained with a conjugate of the invention containing a PNA sequence for inhibiting secretion of gamma-interferon (.gamma.-IFN) by T cells, as detailed in Example 11. As can be seen, the anti-sense PNA conjugate was effective to block .gamma.-IFN secretion when the conjugate was present at levels above about 10 .mu.M. In contrast, no inhibition was seen with the sense-PNA conjugate or the non-conjugated antisense PNA alone.

Detailed Description Text (180):
In another embodiment, the invention is useful for delivering immunospecific antibodies or antibody fragments to the cytosol to interfere with deleterious biological processes such as microbial infection. Recent experiments have shown that intracellular antibodies can be effective antiviral agents in plant and mammalian cells (e.g., Tavladoraki et al. (1993) Nature 366:469; and Shaheen et al. (1996) J. Virol. 70:3392. These methods have typically used single-chain variable region fragments (scFv), in which the antibody heavy and light chains are synthesized as a single polypeptide. The variable heavy and light chains are usually separated by a flexible linker peptide (e.g., of 15 amino acids) to yield a 28 kDa molecule that retains the high affinity ligand binding site. The principal obstacle to wide application of this technology has been efficiency of uptake into infected cells. But by attaching transport polymers to scFv fragments, the degree of cellular uptake can be increased, allowing the immunospecific fragments to bind and disable important microbial components, such as HIV Rev, HIV reverse transcriptase, and integrase proteins.

Detailed Description Paragraph Table (2):
SEQUENCE LISTING <100> GENERAL INFORMATION: <160> NUMBER OF SEQ ID NOS: 57 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 1 <211> LENGTH: 5 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceArg homopolymer R5, Arg oligomer <400> SEQUENCE: 1 Arg Arg Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 2 <211> LENGTH: 6 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceArg homopolymer R6, Arg oligomer <400> SEQUENCE: 2 Arg Arg Arg Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 3 <211> LENGTH: 7 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceArg homopolymer R7, heptamer of L-Arg, hepta-L-Arg, Arg oligomer <400> SEQUENCE: 3 Arg Arg Arg Arg Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 4 <211> LENGTH: 8 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceArg homopolymer R8, Arg oligomer <400> SEQUENCE: 4 Arg Arg Arg Arg Arg Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 5 <211> LENGTH: 9 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceArg homopolymer R9, Arg oligomer <400> SEQUENCE: 5 Arg Arg Arg

Arg Arg Arg Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 6 <211> LENGTH: 7 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequenceheptamer of L-Arg chemical after pH dependent chemical release <400> SEQUENCE: 6 Xaa Arg Arg Arg Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 7 <211> LENGTH: 10 <212> TYPE: PRT PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequenceunlabeled peptide <400> SEQUENCE: 7 Arg Arg Arg Arg Arg Arg Arg Gly Gly Xaa 1 5 10 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 8 8 <211> LENGTH: 9 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequenceanalog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 8 Xaa Lys Lys Arg Arg Gln Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 9 <211> LENGTH: 8 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceTat-49-56 truncated analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 9 Xaa Lys Lys Arg Arg Gln Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 10 <211> LENGTH: 7 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceTat-49-55 truncated analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 10 Xaa Lys Lys Arg Arg Gln Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 11 <211> LENGTH: 8 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceTat-50-57 truncated analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 11 Xaa Lys Arg Arg Gln Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 12 <211> LENGTH: 7 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceTat-51-57 truncated analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 12 Xaa Arg Arg Gln Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 13 <211> LENGTH: 9 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-49 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 13 Xaa Lys Lys Arg Arg Gln Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 14 <211> LENGTH: 9 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-50 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 14 Xaa Ala Lys Arg Arg Gln Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 15 <211> LENGTH: 9 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-51 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 15 Xaa Lys Ala Arg Arg Gln Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 16 <211> LENGTH: 9 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-52 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 16 Xaa Lys Lys Ala Arg Gln Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 17 <211> LENGTH: 9 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-53 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 17 Xaa Lys Lys Arg Ala Gln Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 18 <211> LENGTH: 9 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-54 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 18 Xaa Lys Lys Arg Arg Ala Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 19 <211> LENGTH: 9 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-55 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 19 Xaa Lys Lys Arg Arg Gln Ala Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 20 <211> LENGTH: 9 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-56 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 20 Xaa Lys Lys Arg Arg Gln Arg Ala Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 21 <211> LENGTH: 9 <212> TYPE: PRT <213> ORGANISM:

Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-57 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 21 Xaa Lys Lys Arg Arg Gln Arg Arg Ala 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 22 <211> LENGTH: 9

Detailed Description Paragraph Table (3):
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Arg Arg Xaa Arg Arg Xaa Arg Arg Xaa Arg Arg 1 5 10 15 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 37 <211> LENGTH: 9 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequenceamino acid oligomer delivery enhancing transport moiety <400> SEQUENCE: 37 Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg 1 5 <200> SEQUENCE CHARACTERISTICS: CHARACTERISTICS: <210> SEQ ID NO 38 <211> LENGTH: 11 <212> TYPE: PRT <213> ORGANISM: ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of of Artificial Sequenceamino acid oligomer delivery enhancing transport moiety <400> SEQUENCE: 38 Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg 1 5 10 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 39 <211> LENGTH: 13 <212> TYPE: PRT <213> ORGANISM: ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of of Artificial Sequenceamino acid oligomer delivery enhancing transport moiety <400> SEQUENCE: 39 Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg 1 5 10 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 40 <211> LENGTH: 15 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of of Artificial Sequenceamino acid oligomer delivery enhancing transport moiety <400> SEQUENCE: 40 Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg 1 5 10 15 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 41 <211> LENGTH: 17 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequenceamino acid oligomer delivery enhancing transport moiety <400> SEQUENCE: 41 Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa 1 5 10 15 Arg <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 42 <211> LENGTH: 19 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequenceamino acid oligomer delivery enhancing transport moiety <400> SEQUENCE: 42 Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa 1 5 10 15 Arg Xaa Arg <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 43 <211> LENGTH: 21 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequenceamino acid oligomer delivery enhancing transport moiety <400> SEQUENCE: 43 Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa Arg Xaa 1 5 10 15 Arg Xaa Arg Xaa Arg 20

☐ 3. Document ID: US 6593292 B1

L3: Entry 3 of 13 File: USPT Jul 15, 2003

DOCUMENT-IDENTIFIER: US 6593292 B1
TITLE: Compositions and methods for enhancing drug delivery across and into epithelial tissues

Detailed Description Text (14):
The terms "non-polypeptide agent" and "non-polypeptide therapeutic agent" refer to the portion of a conjugate that does not include the delivery-enhancing transporter, and that is a biologically active agent other than a polypeptide. An example of a non-polypeptide agent is an anti-sense oligonucleotide, which can be conjugated to a poly-arginine peptide to form a conjugate for enhanced delivery into and across one or more layers of an epithelial or endothelial tissue.

Detailed Description Text (23):
The delivery-enhancing transporers increase delivery of the conjugate into and across one or more intact epithelial or endothelial tissue layers compared to delivery of the compound in the absence of the delivery-enhancing transporter. The delivery-enhancing transporters can, in some embodiments, increase delivery of the

conjugate significantly over that obtained using the tat protein of HIV-1 (Frankel et al. (1991) PCT Pub. No. WO 91/09958). Delivery is also increased significantly over the use of shorter fragments of the tat protein containing the tat basic region (residues 49-57 having the sequence RKKRRQRRR; SEQ ID NO:28) (Barsoum et al. (1994) WO 94/04686 and Fawell et al. (1994) Proc. Nat'l. Acad. Sci. USA 91: 664-668). Preferably, delivery obtained using the transporters of the invention is increased more than 2-fold, still more preferably six-fold, still more preferably ten-fold, and still more preferably twenty-fold, over that obtained with tat residues 49-57.

Detailed Description Text (101):
The active compounds of the formulas may be formulated into a suppository comprising, for example, about 0.5% to about 50% of a compound of the invention, disposed in a polyethylene glycol (PEG) carrier (e.g., PEG 1000 [96%] and PEG 4000 [4%]).

Detailed Description Text (148):
The enhanced transport methods of the invention are particularly suited for enhancing transport into and across one or more layers of an epithelial or endothelial tissue for a number of macromolecules, including, but not limited to proteins, nucleic acids, polysaccharides, and analogs thereof. Exemplary nucleic acids include oligonucleotides and polynucleotides formed of DNA and RNA, and analogs thereof, which have selected sequences designed for hybridization to complementary targets (e.g., antisense sequences for single- or double-stranded targets), or for expressing nucleic acid transcripts or proteins encoded by the sequences. Analogs include charged and preferably uncharged backbone analogs, such as phosphonates (preferably methyl phosphonates), phosphoramidates (N3' or N5'), thiophosphates, uncharged morpholino-based polymers, and protein nucleic acids (PNAs). Such molecules can be used in a variety of therapeutic regimens, including enzyme replacement therapy, gene therapy, and anti-sense therapy, for example.

Detailed Description Text (149):
By way of example, protein nucleic acids (PNA) are analogs of DNA in which the backbone is structurally homomorphous with a deoxyribose backbone. The backbone consists of N-(2-aminoethyl)glycine units to which the nucleobases are attached. PNAs containing all four natural nucleobases hybridize to complementary oligonucleotides obeying Watson-Crick base-pairing rules, and is a true DNA mimic in terms of base pair recognition (Egholm et al. (1993) Nature 365:566-568. The backbone of a PNA is formed by peptide bonds rather than phosphate esters, making it well-suited for anti-sense applications. Since the backbone is uncharged, PNA/DNA PNA/DNA or PNA/RNA duplexes that form exhibit greater than normal thermal stability. PNAs have the additional advantage that they are not recognized by nucleases or proteases. In addition, PNAs can be synthesized on an automated peptides synthesizer using standard t-Boc chemistry. The PNA is then readily linked to a transport polymer of the invention.

Detailed Description Text (150):
Examples of anti-sense oligonucleotides whose transport into and across epithelial and endothelial tissues can be enhanced using the methods of the invention are described, for example, in U.S. Pat. No. 5,594,122. Such oligonucleotides are targeted to treat human immunodeficiency virus (HIV). Conjugation of a transport polymer to an anti-sense oligonucleotide can be effected, for example, by forming an an amide linkage between the peptide and the 5'-terminus of the oligonucleotide through a succinate linker, according to well-established methods. The use of PNA conjugates is further illustrated in Example 11 of PCT Application PCT/US98/10571. FIG. 7 of that application shows results obtained with a conjugate of the invention containing a PNA sequence for inhibiting secretion of gamma-interferon (.gamma.-IFN) by T cells, as detailed in Example 11. As can be seen, the anti-sense PNA conjugate was effective to block .gamma.-IFN secretion when the conjugate was present at levels above about 10 .mu.M. In contrast, no inhibition was seen with

the sense-PNA conjugate or the non-conjugated antisense PNA alone.

Detailed Description Text (153):
In another embodiment, the invention is useful for delivering immunospecific antibodies or antibody fragments to the cytosol to interfere with deleterious biological processes such as microbial infection. Recent experiments have shown that intracellular antibodies can be effective antiviral agents in plant and mammalian cells (e.g., Tavladoraki et al. (1993) Nature 366:469; and Shaheen et al. (1996) J. Virol. 70:3392. These methods have typically used single-chain variable region fragments (scFv), in which the antibody heavy and light chains are synthesized as a single polypeptide. The variable heavy and light chains are usually separated by a flexible linker peptide (e.g., of 15 amino acids) to yield a 28 kDa molecule that retains the high affinity ligand binding site. The principal obstacle to wide application of this technology has been efficiency of uptake into infected cells. But by attaching transport polymers to scFv fragments, the degree of cellular uptake can be increased, allowing the immunospecific fragments to bind and disable important microbial components, such as HIV Rev, HIV reverse transcriptase, and integrase proteins.

Detailed Description Paragraph Table (2):
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analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 11 Xaa Lys Lys Arg Arg Gln Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 12 <211> LENGTH: 8 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceTat-51-57 truncated analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 12 Xaa Lys Arg Arg Gln Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 13 <211> LENGTH: 10 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-49 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 13 Xaa Ala Lys Lys Arg Arg Gln Arg Arg Arg 1 5 10 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 14 <211> LENGTH: 10 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-50 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 14 Xaa Arg Ala Lys Arg Arg Gln Arg Arg Arg 1 5 10 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 15 <211> LENGTH: 10 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-51 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 15 Xaa Arg Lys Ala Arg Arg Gln Arg Arg Arg 1 5 10 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 16 <211> LENGTH: 10 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-52 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 16 Xaa Arg Lys Lys Ala Arg Gln Arg Arg Arg 1 5 10 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 17 <211> LENGTH: 10 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-53 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 17 Xaa Arg Lys Lys Arg Ala Gln Arg Arg Arg 1 5 10 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 18 <211> LENGTH: 10 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-54 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 18 Xaa Arg Lys Lys Arg Arg Ala Arg Arg Arg 1 5 10 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 19 <211> LENGTH: 10 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-55 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 19 Xaa Arg Lys Lys Arg Arg Gln Ala Arg Arg 1 5 10 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 20 <211> LENGTH: 10 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-56 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 20 Xaa Arg Lys Lys Arg Arg Gln Arg Ala Arg 1 5 10 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 21 <211> LENGTH: 10 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceA-57 Ala-substituted analog of HIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 21 Xaa Arg Lys Lys Arg Arg Gln Arg Arg Ala 1 5 10 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 22 <211> LENGTH: 10

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Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceArg oligomer R8, Arg homopolymer <400> SEQUENCE: 26 Xaa Arg Arg Arg Arg Arg Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 27 <211> <211> LENGTH: 10 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceArg oligomer R9, Arg homopolymer <400> SEQUENCE: 27 Xaa Arg Arg Arg Arg Arg Arg Arg Arg Arg 1 5 10 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 28 <211> LENGTH: 9 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of of Artificial SequenceHIV-1 tat protein basic region Tat-49-57 <400> SEQUENCE: 28 Arg Lys Lys Arg Arg Gln Arg Arg Arg 1 5 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID ID NO 29 <211> LENGTH: 16 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceAntennapedia homeodomain, Antennapedia-43-58 <400> SEQUENCE: 29 Arg Gln Ile Lys Ile Trp Phe Gln Asn Arg Arg Met Lys Trp Lys Lys 1 5 10 15 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 30 <211> LENGTH: 17 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceAntennapedia homeodomain, Antennapedia-43-58 <400> SEQUENCE: 30 Xaa Arg Gln Ile Lys Ile Trp Phe Gln Asn Arg Arg Met Lys Trp Lys 1 5 10 15 Lys

CLAIMS:

20. The method of claim 1, wherein delivery of the compound into and across the intact epithelial tissue layers is at least two-fold greater than that of the compound conjugated to a basic HIV tat peptide consisting of residues 49-57.

100. The method of claim 61, wherein delivery of the compound into and across the intact epithelial tissue layers is at least two-fold greater than that of the compound conjugated to a basic HIV tat peptide consisting of residues 49-57.

☐ 4. Document ID: US 6495663 B1

L3: Entry 4 of 13 File: USPT Dec 17, 2002

DOCUMENT-IDENTIFIER: US 6495663 B1
TITLE: Method and composition for enhancing transport across biological membranes

Brief Summary Text (10):
The present invention is based in part on the applicants' discovery that conjugation of certain polymers composed of contiguous, highly basic subunits, particularly subunits containing guanidyl or amidinyl moieties, to small molecules or macromolecules is effective to significantly enhance transport of the attached molecule across biological membranes. Moreover, transport occurs at a rate significantly greater than the transport rate provided by a basic HIV tat peptide consisting of residues 49-57 (SEQ ID NO: 1).

Drawing Description Text (8):
FIG. 7 shows inhibition of secretion of gamma-interferon (.gamma.-IFN) by murine T cells as a function of concentration of a sense-PNA-r7 conjugate (SEQ ID NO:18), antisense PNA-r7 conjugate (SEQ ID NO:19), and non-conjugated antisense PNA (SEQ ID NO:20), where the PNA sequences are based on a sequence from the gene for gamma-interferon.

Detailed Description Text (9):
The terms "non-polypeptide agent" and "non-polypeptide therapeutic agent" refer to the portion of a transport polymer conjugate that does not include the transport-enhancing polymer, and that is a biologically active agent other than a polypeptide. An example of a non-polypeptide agent is an anti-sense oligonucleotide, which can be conjugated to a poly-arginine peptide to form a conjugate for enhanced delivery across biological membranes.

Detailed Description Text (61):
FIGS. 1A-1C show results from a study in which polymers of L-arginine (R; FIG. 1A) or D-arginine (r; FIG. 1B) ranging in length from 4 to 9 arginine subunits were tested for ability to transport fluorescein into Jurkat cells. For comparison, transport levels for an HIV tat residues 49-57 ("49-57") and a nonamer of L-lysine (K9) were also tested. FIG. 1C shows a histogram of uptake levels for the conjugates at a concentration of 12.5 .mu.M.

Detailed Description Text (65):
From the foregoing, it is apparent that transport polymers of the invention are significantly more effective than HIV tat peptide 47-59 in transporting drugs across across the plasma membranes of cells. Moreover, the poly-Lys nonamer was ineffective as a transporter.

Detailed Description Text (74):
Experiments carried out in support of the present invention indicate that polymer-facilitated transport is dependent upon metabolic integrity of cells. Addition of a toxic amount of sodium azide (0.5% w/v) to cells resulted in inhibition of uptake of conjugates by about 90% (Example 7). The results shown in FIG. 4 demonstrate (i) sodium azide sensitivity of trans-membrane transport, suggesting energy-dependence (cellular uptake), and (ii) the superiority of poly-guanidinium polymers of the invention (R9, R8, R7) relative to HIV tat(49-57).

Detailed Description Text (101):
The enhanced transport method of the invention is particularly suited for enhancing transport across biological membranes for a number of macromolecules, including, but not limited to proteins, nucleic acids, polysaccharides, and analogs thereof. Exemplary nucleic acids include oligonucleotides and polynucleotides formed of DNA and RNA, and analogs thereof, which have selected sequences designed for hybridization to complementary targets (e.g., antisense sequences for single- or double-stranded targets), or for expressing nucleic acid transcripts or proteins encoded by the sequences. Analogs include charged and preferably uncharged backbone analogs, such as phosphonates (preferably methyl phosphonates), phosphoramidates (N3' or N5'), thiophosphates, uncharged morpholino-based polymers, and protein nucleic acids (PNAs). Such molecules can be used in a variety of therapeutic regimens, including enzyme replacement therapy, gene therapy, and anti-sense therapy, for example.

Detailed Description Text (102):
By way of example, protein nucleic acids (PNA) are analogs of DNA in which the backbone is structurally homomorphous with a deoxyribose backbone. It consists of N-(2-aminoethyl)glycine units to which the nucleobases are attached. PNAs containing all four natural nucleobases hybridize to complementary oligonucleotides obeying Watson-Crick base-pairing rules, and is a true DNA mimic in terms of base pair recognition (Egholm et al., 1993). The backbone of a PNA is formed by peptide bonds rather than phosphate esters, making it well-suited for anti-sense applications. Since the backbone is uncharged, PNA/DNA or PNA/RNA duplexes that form exhibit greater than normal thermal stability. PNAs have the additional advantage that they are not recognized by nucleases or proteases. In addition, PNAs can be synthesized on an automated peptides synthesizer using standard t-Boc chemistry. The PNA is then readily linked to a transport polymer of the invention.

Detailed Description Text (103):
Examples of anti-sense oligonucleotides whose transport into cells may be enhanced using the methods of the invention are described, for example, in U.S. Pat. No. 5,594,122. Such oligonucleotides are targeted to treat human immunodeficiency virus (HIV). Conjugation of a transport polymer to an anti-sense oligonucleotide can be effected, for example, by forming an amide linkage between the peptide and the 5'-terminus of the oligonucleotide through a succinate linker, according to well-established methods. The use of PNA conjugates is further illustrated in Example 11.

Detailed Description Text (104):
FIG. 7 shows results obtained with a conjugate of the invention containing a PNA sequence for inhibiting secretion of gamma-interferon (.gamma.-IFN) by T cells, as detailed in Example 11. As can be seen, the anti-sense PNA conjugate was effective to block .gamma.-IFN secretion when the conjugate was present at levels above about 10 .mu.M. In contrast, no inhibition was seen with the sense-PNA conjugate or the non-conjugated antisense PNA alone.

Detailed Description Text (110):
In another embodiment, the invention is useful for delivering immunospecific antibodies or antibody fragments to the cytosol to interfere with deleterious biological processes such as microbial infection. Recent experiments have shown that intracellular antibodies can be effective antiviral agents in plant and mammalian cells (e.g., Tavladoraki et al., 1993; and Shaheen et al., 1996). These methods have typically used single-chain variable region fragments (scFv), in which the antibody heavy and light chains are synthesized as a single polypeptide. The variable heavy and light chains are usually separated by a flexible linker peptide (e.g., of 15 amino acids) to yield a 28 kDa molecule that retains the high affinity ligand binding site. The principal obstacle to wide application of this technology has been efficiency of uptake into infected cells. But by attaching transport polymers to scFv fragments, the degree of cellular uptake can be increased, allowing the immunospecific fragments to bind and disable important microbial components, such as HIV Rev, HIV reverse transcriptase, and integrase proteins.

Detailed Description Text (132):
The active compounds of the formulas may be formulated into a suppository comprising, for example, about 0.5% to about 50% of a compound of the invention, disposed in a polyethylene glycol (PEG) carrier (e.g., PEG 1000 [96%] and PEG 4000 [4%]).

Detailed Description Text (149):
Uptake levels of the following polypeptides were measured by the method in Example 2: (1) a polypeptide comprising HIV tat residues 49-57 (Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg=SEQ ID NO:1), (2) a nonamer of L-Lys residues (K9, SEQ ID NO:2), and (3) homo-L or homo- polypeptides containing four to nine Arg residues (SEQ ID NO:3-8 and 12-17). Results are shown in FIGS. 2A-2C.

Detailed Description Text (195):
1. PNA-Peptide Conjugates. The following sense and antisense PNA-peptide conjugates were prepared for inhibiting gamma-IFN production, where r=D-arginine, and R=L-arginine:

Detailed Description Text (197):
Antisense: NH.sub.2 -rrrrrrr-GTGTAGCGTT-COOH (SEQ ID NO:19)

Detailed Description Text (198):
Fluorescent antisense: X-rrrrrrr-GTGTAGCGTT-COOH (X-SEQ ID NO:19) where X=fluorescein-aminocaproate

Detailed Description Text (199):
Biotinylated antisense: Z-rrrrrrr-GTGTAGCGTT-COOH (Z-SEQ ID NO:19) where Z=biotin-aminocaproate

Detailed Description Text (200):
2. Uptake by T Cells. To show that PNA-polyarginine conjugates enter cells effectively, the fluorescent antisense conjugate above (X-SEQ ID NO:19) was synthesized by conjugating fluorescein isothiocyanate to the amino terminus of SEQ ID NO:18 using an aminocaproic acid spacer.

Detailed Description Text (201):
Cellular uptake was assayed by incubating the Jurkat human T cell line (5.times.10.sup.5 cells/well) either pretreated for minutes with 0.5% sodium azide or phosphate buffered saline, with varying amounts (100 nM to 50 .mu.M) of the fluorescein-labeled sense and antisense PNA-r7 conjugate, as well as the antisense PNA alone (without r7 segment). The amount of antisense PNA that entered the cells was analyzed by confocal microscopy and FACS. In both cases, fluorescent signals were present only in cells not exposed to azide, and the fluorescent signal was dependent on the dose of the fluorescent conjugate and on the temperature and duration of incubation.

Detailed Description Text (205):
After 24 hours, aliquots of the cultured supernatants were taken, and the amount of gamma-IFN was measured using the fluorescent binding assay described in section 3 above. Treatment of cells with the antisense PNA-r7 conjugate resulted in an over 70% reduction in IFN secretion, whereas equivalent molar amounts of the sense PNA-r7, antisense PNA lacking r7, or r7 alone all showed no inhibition (FIG. 7).

☐ 5. Document ID: US 6348583 B1

L3: Entry 5 of 13 File: USPT Feb 19, 2002

DOCUMENT-IDENTIFIER: US 6348583 B1
**** See image for Certificate of Correction ****
TITLE: Poly(ether-thioether), poly(ether-sulfoxide) and poly(ether-sulfone) nucleic acids

Brief Summary Text (2):
The present invention relates, in general, to nucleotide mimetics and their derived nucleic acid mimetics, methods for the construction of both and the use of the nucleic acid mimetics in biochemistry and medicine. More particularly, the present invention relates to (i) acyclic nucleotide mimetics, also referred to as acyclic nucleotides, based upon a poly(ether-thioether), poly(ether-sulfoxide) or poly (ether-sulfone) backbone; (ii) a method for synthesizing the acyclic nucleotide mimetics; (iii) acyclic nucleotide mimetic sequences, also referred to as acyclic polynucleotide sequences; (iv) a method for synthesizing the acyclic nucleotide mimetic sequences; and (v) use of the acyclic nucleotide mimetic sequences as oligonucleotides in, for example, antisense applications and procedures.

Brief Summary Text (3):
An antisense oligonucleotide (e.g., antisense oligodeoxyribonucleotide) may bind its its target nucleic acid either by Watson-Crick base pairing or Hoogsteen and anti-Hoogsteen base pairing. To this effect see, Thuong and Helene (1993) Sequence

specific recognition and modification of double helical DNA by oligonucleotides Angev. Chem. Int. Ed. Engl. 32:666. According to the Watson-Crick base pairing, heterocyclic bases of the antisense oligonucleotide form hydrogen bonds with the heterocyclic bases of target single-stranded nucleic acids (RNA or single-stranded DNA), whereas according to the Hoogsteen base pairing, the heterocyclic bases of the target nucleic acid are double-stranded DNA, wherein a third strand is accommodated in the major groove of the B-form DNA duplex by Hoogsteen and anti-Hoogsteen base pairing to form a triplex structure.

Brief Summary Text (4):
According to both the Watson-Crick and the Hoogsteen base pairing models, antisense oligonucleotides have the potential to regulate gene expression and to disrupt the essential functions of the nucleic acids. Therefore, antisense oligonucleotides have possible uses in modulating a wide range of diseases.

Brief Summary Text (7):
Gene expression involves few distinct and well-regulated steps. The first major step of gene expression involves transcription of a messenger RNA (mRNA) which is an RNA sequence complementary to the antisense (i.e., -) DNA strand, or, in other words, identical in sequence to the DNA sense (i.e., +) strand, composing the gene. In eukaryotes, transcription occurs in the cell nucleus.

Brief Summary Text (10):
There is also evidence that in some cases gene expression is downregulated by endogenous antisense RNA repressors that bind a complementary mRNA transcript and thereby prevent its translation into a functional protein. To this effect see Green et al. (1986) The role of antisense RNA in gene regulation. Ann. Rev. Biochem. 55:569.

Brief Summary Text (17):
At the transcription level, antisense or sense oligonucleotides or analogs that bind bind to the genomic DNA by strand displacement or the formation of a triple helix, may prevent transcription. To this effect see, Thuong and Helene (1993) Sequence specific recognition and modification of double helical DNA by oligonucleotides Angev. Chem. Int. Ed. Engl. 32:666.

Brief Summary Text (18):
At the transcript level, antisense oligonucleotides or analogs that bind target mRNA mRNA molecules lead to the enzymatic cleavage of the hybrid by intracellular RNase H. To this effect see Dash et al. (1987) Proc. Natl. Acad. Sci. USA, 84:7896. In this case, by hybridizing to the targeted mRNA, the oligonucleotides or oligonucleotide analogs provide a duplex hybrid recognized and destroyed by the RNase H enzyme. Alternatively, such hybrid formation may lead to interference with correct splicing. To this effect see Chiang et al. (1991) Antisense oligonucleotides inhibit intercellular adhesion molecule 1 expression by two distinct mechanisms. J. Biol. Chem. 266:18162. As a result, in both cases, the number of the target mRNA intact transcripts ready for translation is reduced or eliminated.

Brief Summary Text (19):
At the translation level, antisense oligonucleotides or analogs that bind target mRNA molecules prevent, by steric hindrance, binding of essential translation factors (ribosomes), to the target mRNA, as described by Paterson et al. (1977) Proc. Natl. Acad. Sci. USA, 74:4370, a phenomenon known in the art as hybridization arrest, disabling the translation of such mRNAs.

Brief Summary Text (20):
Thus, antisense sequences, which as described hereinabove, may arrest the expression expression of any endogenous and/or exogenous gene depending on their specific sequence, attracted much attention by scientists and pharmacologists who were

devoted at developing the antisense approach into a new pharmacological tool. To this effect see Cohen (1992) Oligonucleotide therapeutics. Trends in Biotechnology, 10:87.

Brief Summary Text (21):
For example, several antisense oligonucleotides have been shown to arrest hematopoietic cell proliferation (Szczylik et al (1991) Selective inhibition of leukemia cell proliferation by BCR-ABL antisense oligodeoxynucleotides. Science 253:562), growth (Calabretta et al. (1991) Normal and leukemic hematopoietic cell manifest differential sensitivity to inhibitory effects of c-myc antisense oligodeoxynucleotides: an in vitro study relevant to bone marrow purging. Proc. Natl. Acad. Sci. USA 88:2351), entry into the S phase of the cell cycle (Heikhila et al. (1987) A c-myc antisense oligodeoxynucleotide inhibits entry into S phase but but not progress from G(0) to G(1). Nature, 328:445), reduced survival (Reed et al. (1990) Antisense mediated inhibition of BCL2 prooncogene expression and leukemic cell growth and survival: comparison of phosphodiester and phosphorothioate oligodeoxynucleotides. Cancer Res. 50:6565) and prevent receptor mediated responses (Burch and Mahan (1991) Oligodeoxynucleotides antisense to the interleukin I receptor m RNA block the effects of interleukin I in cultured murine and human fibroblasts and in mice. J. Clin. Invest. 88:1190). For use of antisense oligonucleotides as antiviral agents the reader is referred to Agrawal (1992) Antisense oligonucleotides as antiviral agents. TIBTECH 10:152.

Brief Summary Text (22):
For efficient in vivo inhibition of gene expression using antisense oligonucleotides or analogs, the oligonucleotides or analogs must fulfill the following requirements (i) sufficient specificity in binding to the target sequence; (ii) solubility in water; (iii) stability against intra- and extracellular nucleases; (iv) capability of penetration through the cell membrane; and (v) when used to treat an organism, low toxicity.

Brief Summary Text (23):
Unmodified oligonucleotides are impractical for use as antisense sequences since they have short in vivo half-lives, during which they are degraded rapidly by nucleases. Furthermore, they are difficult to prepare in more than milligram quantities. In addition, such oligonucleotides are poor cell membrane penetraters, see, Uhlmann et al. (1990) Chem. Rev. 90:544.

Brief Summary Text (27):
Oligonucleotides can be modified either in the base, the sugar or the phosphate moiety. These modifications include the use of methylphosphonates, monothiophosphates, dithiophosphates, phosphoramidates, phosphate esters, bridged phosphorothioates, bridged phosphoramidates, bridged methylenephosphonates, dephospho internucleotide analogs with siloxane bridges, carbonate bridges, carboxymethyl ester bridges, acetamide bridges, carbamate bridges, thioether bridges, sulfoxy bridges, sulfono bridges, various "plastic" DNAs, .alpha.-anomeric bridges and borane derivatives. For further details the reader is referred to Cook (1991) Medicinal chemistry of antisense oligonucleotides--future opportunities. Anti-Cancer Drug Design 6:585.

Brief Summary Text (44):
There is thus a widely recognized need for, and it would be highly advantageous to have, oligonucleotide analogs devoid of these drawbacks, and which are characterized by (i) ease of synthetic procedure and proven synthetic efficiency; and which are further characterized by properties common to the above described polyether nucleic acids, such as (ii) sufficient specificity in binding to target sequences; (iii) solubility in water; (iv) stability against intra- and extracellular nucleases; (v) capability of penetrating through cell membranes; and (vi) when used to treat an organism, low toxicity, properties that collectively render an oligonucleotide analog highly suitable as an antisense therapeutic drug.

Brief Summary Text (102):
The present invention successfully addresses the shortcomings of the presently known configurations by providing an oligonucleotide analog characterized by (i) sufficient specificity in binding its target sequence; (ii) solubility in water; (iii) stability against intra- and extracellular nucleases; (iv) capability of penetrating through the cell membrane; and (v) when used to treat an organism, low toxicity, properties collectively rendering the oligonucleotide analog of the present invention highly suitable as an antisense therapeutic is drug, and, above all being readily sythesizable.

Detailed Description Text (2):
The present invention is of compounds that are not polynucleotides yet which bind to complementary DNA and RNA sequences, the compounds according to the present invention include naturally occurring nucleobases or other nucleobases binding moieties (also referred herein as nucleobase analogs) covalently bound to a poly (ether-thioether), a poly(ether-sulfoxide) and/or a poly(ether-sulfone) backbone, which can be used as oligonucleotide analogs in, for example, antisense applications and procedures. The oligonucleotide analogs according to the present invention include a new acyclic biopolymer backbone which best fulfills the six criteria for selecting antisense oligonucleotide analogs listed in the Background section above.

Detailed Description Text (3):
The synthesis, structure and mode of operation of antisense oligonucleotide analogs according to the present invention may be better understood with reference to the drawings and accompanying descriptions.

Detailed Description Text (51):
The present invention is further directed at therapeutic and/or prophylactic uses for poly(ether-thioether), poly(ether-sulfoxide) or poly(ether-sulfone) nucleic acids. Likely therapeutic and prophylactic targets according to the invention include but are not limited to human papillomavirus (HPV), herpes simplex virus (HSV), candida albicans, influenza virus, human lo immunodeficiency virus (HIV), intracellular adhesion molecules (ICAM), cytomegalovirus (CMV), phospholipase A2 (PLA2), 5-lipoxygenase (5-LO), protein kinase C (PKC), and RAS oncogene.

Detailed Description Text (64):
Second, one of the major drawbacks of PNAs when used as antisense molecules is that PNA-DNA hybrids are characterized by high melting temperature (Tm). For example, the Tm value for a duplex such as PNA-T.sub.10 -dA.sub.10 is greater than 70.degree. C., whereas the Tm value of the equivalent native double stranded DNA (dT.sub.10 -dA.sub.10) (SEQ ID NO:18) is nearly three fold lower, about 24.degree. C. Because PNAs bind complementary sequences so strongly, at body temperature (e.g., 37.degree. C.) PNAs lack the specificity to their intended counterparts and end up binding not just to target sequences but also to other strands of DNA, RNA, or even proteins, incapacitating the cell in unforeseen ways. PNAs act as a micelle when the lysine residues are solvated. PNAs are poorly miscible in water, while the hydrophobic nature of the backbone have a tendency to seek for a nonpolar environment e.g., the bases of the natural complementary DNA. These hydrophobic interactions are the major driving force for the formation of highly stable PNA-DNA hybrid and therefore very high Tm values for such hybrids. The unique solubility nature of poly(ether-thioether), poly(ether-sulfoxide) or poly(ether-sulfone) nucleic acids, by conserving the hydrophobic-hydrophilic properties of polyethers such as PEG, yield Tm values slightly higher than natural DNA, yet much lower values than PNAs, which moderate values are of great importance for specificity.

Detailed Description Text (66):
Fourth, PEG is approved by the FDA for parenteral use, topical application, and as a a constituent of suppositories, nasal sprays, foods and cosmetics. PEG is of low

toxicity when administered orally or parenterally, and only large quantities involve involve adverse reactions. See, Smyth, H. F. et al. (1955) J. Am. Pharm. Assoc., 34:27. Evidences accumulated experiencing administration of PEG-protein conjugates, suggest that both the plasma half-lives (circulating time) of PEG conjugated proteins and their bioavailability improves as compared with the native proteins, which improvement is accompanied by improved efficacy. Ganser et al. (1989) Blood, 73:31, observed less side effects at lower dosage using PEG-modifications. Reduced toxicity has been observed with several PEG-modified enzymes, see Fuertges et al. (1990) J. Contr. Release, 11:139. Another advantage in exploiting the improved pharmacokinetics of PEG is the option of administrating bolus injections instead of continuous intravenous infusions, as described by Pizzo (1991) Adv. Drug Del. Rev. 6:153. In the preferred embodiments of the invention, poly(ether-thioether) nucleic acids include a PEG backbone and/or are conjugated to PEG exoconjugates and therefore enjoy the above listed advantages.

Detailed Description Text (136):
Such cycles can be repeated as much as needed to form appropriate sense or antisense sequence, wherein in each tri-stages cycle, one additional monomer is sequentially added to the growing chain, up to the desired n cycle.

☐ 6. Document ID: US 6306993 B1

L3: Entry 6 of 13 File: USPT Oct 23, 2001

DOCUMENT-IDENTIFIER: US 6306993 B1
TITLE: Method and composition for enhancing transport across biological membranes

Brief Summary Text (32):
Frankel et al. (1991) reported that conjugating selected molecules to the tat protein of HIV can increase cellular uptake of those molecules. However, use of the tat protein has certain disadvantages, including unfavorable aggregation and insolubility properties.

Brief Summary Text (35):
The present invention is based in part on the applicants' discovery that conjugation of certain polymers composed of contiguous, highly basic subunits, particularly subunits containing guanidyl or amidinyl moieties, to small molecules or macromolecules is effective to significantly enhance transport of the attached molecule across biological membranes. Moreover, transport occurs at a rate significantly greater than the transport rate provided by a basic HIV tat peptide consisting of residues 49-57.

Drawing Description Text (10):
FIG. 7 shows inhibition of secretion of gamma-interferon (.gamma.-IFN) by murine T cells as a function of concentration of a sense-PNA-r7 conjugate (SEQ ID NO: 6 conjulated to), antisense PNA-r7 conjugate (SEQ ID NO: 6 conjulated to), and non-conjugated antisense PNA (SEQ ID NO: 19), where the PNA sequences are based on a sequence from the gene for gamma-interferon.

Detailed Description Text (9):
The terms "non-polypeptide agent" and "non-polypeptide therapeutic agent" refer to the portion of a transport polymer conjugate that does not include the transport-enhancing polymer, and that is a biologically active agent other than a

polypeptide. An example of a non-polypeptide agent is an anti-sense oligonucleotide, oligonucleotide, which can be conjugated to a poly-arginine peptide to form a conjugate for enhanced delivery across biological membranes.

Detailed Description Text (62):
FIGS. 1A-1C show results from a study in which polymers of L-arginine (R; FIG. 1A) or D-arginine (r; FIG. 1B) ranging in length from 4 to 9 arginine subunits were tested for ability to transport fluorescein into Jurkat cells. For comparison, transport levels for an HIV tat residues 49-57 ("49-57") and a nonamer of L-lysine (K9) were also tested. FIG. 1C shows a histogram of uptake levels for the conjugates at a concentration of 12.5 .mu.M.

Detailed Description Text (66):
From the foregoing, it is apparent that transport polymers of the invention are significantly more effective than HIV tat peptide 47-59 in transporting drugs across across the plasma membranes of cells. Moreover, the poly-Lys nonamer was ineffective as a transporter.

Detailed Description Text (75):
Experiments carried out in support of the present invention indicate that polymer-facilitated transport is dependent upon metabolic integrity of cells. Addition of a toxic amount of sodium azide (0.5% w/v) to cells resulted in inhibition of uptake of conjugates by about 9% (Example 7). The results shown in FIG. 4 demonstrate (i) sodium azide sensitivity of trans-membrane transport, suggesting energy-dependence (cellular uptake), and (ii) the superiority of poly-guanidinium polymers of the invention (R9, R8, R7) relative to HIV tat(49-57).

Detailed Description Text (102):
The enhanced transport method of the invention is particularly suited for enhancing transport across biological membranes for a number of macromolecules, including, but not limited to proteins, nucleic acids, polysaccharides, and analogs thereof. Examplary nucleic acids include oligonucleotides and polynucleotides formed of DNA and RNA, and analogs thereof, which have selected sequences designed for hybridization to complementary targets (e.g., antisense sequences for single- or double-stranded targets), or for expressing nucleic acid transcripts or proteins encoded by the sequences. Analogs include charged and preferably uncharged backbone analogs, such as phosphonates (preferably methyl phosphonates), phosphoramidates (N3' or N5'), thiophosphates, uncharged morpholino-based polymers, and protein nucleic acids (PNAs). Such molecules can be used in a variety of therapeutic regimens, including enzyme replacement therapy, gene therapy, and anti-sense therapy, for example.

Detailed Description Text (103):
By way of example, protein nucleic acids (PNA) are analogs of DNA in which the backbone is structurally homomorphous with a deoxyribose backbone. It consists of N-(2-aminoethyl)glycine units to which the nucleobases are attached. PNAs containing all four natural nucleobases hybridize to complementary oligonucleotides obeying Watson-Crick base-pairing rules, and is a true DNA mimic in terms of base pair recognition (Egholm et al., 1993). The backbone of a PNA is formed by peptide bonds rather than phosphate esters, making it well-suited for anti-sense applications. Since the backbone is uncharged, PNA/DNA or PNA/RNA duplexes that form exhibit greater than normal thermal stability. PNAs have the additional advantage that they are not recognized by nucleases or proteases. In addition, PNAs can be synthesized on an automated peptides synthesizer using standard t-Boc chemistry. The PNA is then readily linked to a transport polymer of the invention.

Detailed Description Text (104):
Examples of anti-sense oligonucleotides whose transport into cells may be enhanced using the methods of the invention are described, for example, in U.S. Pat. No. 5,594,122. Such oligonucleotides are targeted to treat human immunodeficiency virus

(HIV). Conjugation of a transport polymer to an anti-sense oligonucleotide can be effected, for example, by forming an amide linkage between the peptide and the 5'-terminus of the oligonucleotide through a succinate linker, according to well-established methods. The use of PNA conjugates is further illustrated in Example 11.

Detailed Description Text (105):
FIG. 7 shows results obtained with a conjugate of the invention containing a PNA sequence for inhibiting secretion of gamma-interferon (.gamma.-IFN) by T cells, as detailed in Example 11. As can be seen, the anti-sense PNA conjugate was effective to block .gamma.-IFN secretion when the conjugate was present at levels above about 10 .mu.M. In contrast, no inhbition was seen with the sense-PNA conjugate or the non-conjugated antisense PNA alone.

Detailed Description Text (111):
In another embodiment, the invention is useful for delivering immunospecific antibodies or antibody fragments to the cytosol to interfere with deleterious biological processes such as microbial infection. Recent experiments have shown that intracellular antibodies can be effective antiviral agents in plant and mammalian cells (e.g., Tavladoraki et al., 1993; and Shaheen et al., 1996). These methods have typically used single-chain variable region fragments (scFv), in which the antibody heavy and light chains are synthesized as a single polypeptide. The variable heavy and light chains are usually separated by a flexible linker peptide (e.g., of 15 amino acids) to yield a 28 kDa molecule that retains the high affinity ligand binding site. The principal obstacle to wide application of this technology has been efficiency of uptake into infected cells. But by attaching transport polymers to scFv fragments, the degree of cellular uptake can be increased, allowing the immunospecific fragments to bind and disable important microbial components, such as HIV Rev, HIV reverse transcriptase, and integrase proteins.

Detailed Description Text (133):
The active compounds of the formulas may be formulated into a suppository comprising, for example, about 0.5% to about 50% of a compound of the invention, disposed in a polyethylene glycol (PEG) carrier (e.g., PEG 1000 [96%] and PEG 4000 [4%]).

Detailed Description Text (150):
Uptake levels of the following polypeptides were measured by the method in Example 2: (1) a polypeptide comprising HIV tat residues 49-57 (Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg=SEQ ID NO:1), (2) a nonamer of L-Lys residues (K9, SEQ ID NO:2), and (3) homo-L or homo-D polypeptides containing four to nine Arg residues (SEQ ID NO:3-8 and 12-17). Results a re shown in FIGS. 2A-2C.

Detailed Description Text (194):
1. PNA-Peptide Conjugates. The following sense and antisense PNA-peptide conjugates were prepared for inhibiting gamma-IFN production, where r=D-arginine, and R=L-arginine:

Detailed Description Text (197):
Antisense:

Detailed Description Text (199):
Fluorescent antisense:

Detailed Description Text (202):
Biotinylated antisense:

Detailed Description Text (205):
2. Uptake by T Cells. To show that PNA-polyarginine conjugates enter cells effectively, the fluorescent antisense conjugate above (XSEQ ID NO:6 conjugated to

SEQ ID NO:19) was synthesized by conjugating fluorescein isothiocyanate to the amino amino terminus of r7 the above conjugated with SEQ ID NO:18 using an aminocaproic acid spacer.

Detailed Description Text (206):
Cellular uptake was assayed by incubating the Jurkat human T cell line (5.times.10.sup.5 cells/well) either pretreated for 30 minutes with 0.5% sodium azide or phosphate buffered saline, with varying amounts (100 nM to 50 .mu.M) of the fluorescein-labeled sense and antisense PNA-r7 conjugate, as well as the antisense PNA alone (without r7 segment). The amount of antisense PNA that entered the cells was analyzed by confocal microscopy and FACS. In both cases, fluorescent signals were present only in cells not exposed to azide, and the fluorescent signal was dependent on the dose of the fluorescent conjugate and on the temperature and duration of incubation.

Detailed Description Text (209):
After 24 hours, aliquots of the cultured supernatants were taken, and the amount of gamma-IFN was measured using the fluorescent binding assay described in section 3 above. Treatment of cells with the antisense PNA-r7 conjugate resulted in an over 70% reduction in IFN secretion, whereas equivalent molar amounts of the sense PNA-r7, antisense PNA lacking r7, or r7 alone all showed no inhibition (FIG. 7).

☐ 7. Document ID: US 6168784 B1

L3: Entry 7 of 13 File: USPT Jan 2, 2001

DOCUMENT-IDENTIFIER: US 6168784 B1
TITLE: N-terminal modifications of RANTES and methods of use

Abstract Text (1):
N-terminally modified RANTES derivatives are disclosed. The derivatives effectively block the inflammatory effects of RANTES, and are useful for the treatment of asthma, allergic rhinitis, atopic dermatitis, atheroma/atherosclerosis, and rheumatoid arthritis. Additionally, the compounds are useful for the treatment of HIV infection.

Brief Summary Text (2):
The invention relates to N-terminally modified RANTES derivatives that effectively lock the inflammatory effects of RANTES, and are thus useful for the treatment of asthma, allergic rhinitis, atopic dermatitis, atheroma/atheroschleosis, and rheumatoid arthritis. Additionally, the compounds are useful for the treatment of human immune deficiency virus ("HIV").

Brief Summary Text (5):
Chemokines have the ability to recruit and activate a wide variety of proinflammatory cell types, and RANTES has been shown to elicit an inflammatory response in vivo. RANTES, along with the natural ligands for the CCR5 chemokine receptor, MIP-1.alpha., MIP-1.beta., were found to inhibit human immune deficiency virus type-1 ("HIV-1") infection (Cocchi, et al., Science 270:1811-1815 (1995)), leading to the identification of CCR5 as the major co-receptor for primary isolates of HIV-1, HIV-2 and SIV-1 (Deng, et al., Nature 381:661-666 (1996); Doranz, et al., Cell 85:1149-1158 (1996); Choe, et al., Cell 85:1135-1148 (1996); Chen, et al., J. Virol. 71:2705-2714 (1997); and Alkhatib, et al., Science 272:1955-1958 (1996)).

However, although RANTES consistently inhibits HIV-1 replication in peripheral blood blood mononuclear cells, it does not block infection of primary macrophage cultures, which suggests that RANTES would not influence HIV replication in non-lymphocyte cell types.

Brief Summary Text (6):
N-terminal modifications of RANTES result in antagonists that can block HIV-1 infection without signaling calcium flux (Mack, et al., J. Exp. Med. 187:1215-1224 (1998) and Proudfoot, et al., J. Biol. Chem. 271:2599-2603 (1996)). These modifications include N-terminal truncation [RANTES 9-68] (Arenzana-Seisdedos, et al., Nature 383:400 (1996)), and addition of methionine ("Met-RANTES") or aminooxypentane ("AOP-RANTES") at the N-terminus of RANTES (Mack, et al., supra and Simmons, et al., Science 276:276-279 (1997)). It has been reported that the Met-RANTES and AOP-RANTES derivatives are antagonists of RANTES. Further, N-terminally modified RANTES, with a higher affinity for CCR5 than native RANTES are more potent than native RANTES in blocking infection (Simmons, et al., supra).

Brief Summary Text (7):
Chemokine receptor antagonists that are potent, selective, and achieve full receptor occupancy would clearly be useful for the treatment of HIV-1 in infected individuals. Surprisingly, compounds have been discovered with this spectrum of activity. These derivatives inhibited infection of many different cell types, including macrophages and lymphocytes.

Brief Summary Text (14):
Also provided are pharmaceutical compositions and methods of treating disease states, including HIV infection, by administering therapeutically effective amounts of compounds comprising RANTES derivatives, or pharmaceutically acceptable salts thereof.

Drawing Description Text (3):
FIG. 2 shows the effect of AOP-RANTES treatment on HIV infection in hu-PBL-SCID mice.

Drawing Description Text (4):
FIG. 3 depicts the partial sequences of several HIV-1 clones cons (SEQ ID NO:7), 242 (SEQ ID NO:8), 242/H (SEQ ID NO:9), 241 (SEQ ID NO:10), 230 (SEQ ID NO:11) and mouse 1-4 (SEQ ID NO:9).

Drawing Description Text (6):
FIG. 5 depicts the effect of AOP-RANTES on HIV-1 infection in PBMC cultures.

Drawing Description Text (7):
FIG. 6 shows the effect of n-nonanoyl-RANTES (2-68) (SEQ ID NO:2) on HIV-1 infection.

Drawing Description Text (8):
FIG. 7 shows the inhibition of HIV-1 infection by AOP- and NNY-RANTES in cultured primary human PBMC. Virus replication was measured by p24 capsid antigen production after 5-7 days of infection. FIG. 7A shows infection with the R5 SF162 isolate and FIG. 7B shows infection with two R.5 variants of HIV-1 242.

Drawing Description Text (9):
FIG. 8 shows inhibition of HIV-1 infection by AOP- or NNY-RANTES in hu-PBL-SCID mice. CCR5 antagonists were delivered by subcutaneously implanted osmotic pumps at the rate of 2.5 .mu.g/hr beginning 1 day before HIV-1 infection. A single dose of 1 mg of AOP-RANTES (FIG. 8A) or NNY-RANTES (FIGS. 8B and 8C) was administered just prior to HIV-1 challenge. Data presented are plasma HIV RNA copies/ml at 1-4 weeks after infection, and each point represents the value for a single animal. The horizontal bar in each panel represents the duration of continuous AOP- or NNY-

RANTES administration.

Detailed Description Text (18):
M-tropic HIV viruses, also referred to as "R5" viruses, enter cells via the CD4 and CCR5 receptors. T-tropic viruses, also referred to as "X4" viruses, enter cells via the CD4 and CXCR4 receptors. Dual-tropic viruses, also referred to as "R5.times.4" viruses, mediate entry via more than one of the co-receptors. For example, HIV-1 242 is an M-tropic or R5 virus. HIV-1 230 is a T-tropic or X4 virus. HIV-1 241 is a dual-tropic or R5.times.4 virus. Chesebro, et al., J. Virol. 70:9055-9059 (1996); Speck, et al., J. Virol. 71:7136-7139 (1997). For nomenclature classifications of 25 HIV-1 isolates by co-receptor, see Berger, et al., Nature 391:240 (1998).

Detailed Description Text (19):
A preferred use of the polypeptides of the present invention is in inhibiting HIV-1 infection in mammals.

Detailed Description Text (25):
As used herein, the term "therapeutically effective amount" refers to that amount of a polypeptide of the invention which, when administered to a mammal in need thereof, is sufficient to effect treatment (as defined above), for example, as an anti-inflammatory agent, anti-asthmatic agent, an immunosuppressive agent, or anti-autoimmune disease agent to inhibit HIV-1 infection in mammals. The amount that constitutes a "therapeutically effective amount" will vary depending on the polypeptide, the condition or disease and its severity, and the mammal to be treated, its weight, age, etc., but may be determined routinely by one of ordinary skill in the art with regard to contemporary knowledge and to this disclosure.

Detailed Description Text (39):
The compounds of the present invention have been found to possess valuable pharmacological properties, and have been shown to effectively block the inflammatory effects of RANTES. Accordingly, they are useful for the treatment of asthma, allergic rhinitis, atopic dermatitis, atheroma/atheroschleosis, and rheumatoid arthritis. The compounds of the present invention have also been shown to inhibit HIV-1 infection in vitro.

Detailed Description Text (41):
The potential of the compounds for utility against HIV-1 is determined by the method, described in the following Examples. The potential of the compounds for utility against inflammatory effects is determined by methods well known to those skilled in the art.

Detailed Description Text (57):
Another method of administering the polypeptides of the invention utilizes both a bolus injection and a continuous infusion. This is a particularly preferred method when the therapeutic treatment is for the prevention of HIV-1 infection.

Detailed Description Text (61):
For systemic administration via suppository, traditional binders and carriers include, for example, polyethylene glycols or triglycerides, for example PEG 1000 (96%) and PEG 4000 (4%). Such suppositories may be formed from mixtures containing the active ingredient in the range of from about 0.5 w/w % to about 10 w/w %; preferably from about 1 w/w % to about 2 w/w %.

Detailed Description Text (101):
HIV-1 Inhibition Assays

Detailed Description Text (102):
A. Chemokine receptors act as co-factors for HIV-1 entry into CD4.sup.+ cells. The CC chemokines MIP-1.alpha., MIP-1.beta., RANTES and eotaxin can suppress some strains of HIV replication in primary peripheral blood mononuclear cells ("PBMCs")

and chemokine receptor transfected cell lines. The viral produced chemokine vMIP-1 inhibits some primary non-syncytium inducing (NSI) HIV strains when co-transfected with the NSI strain HIV-1 co-receptor CCR5. CCR3 is the predominant chemokine receptor through which eotaxin, RANTES and other CC chemokines activate eosinophils. RANTES (SEQ ID NO:1) and MIP-1.alpha. also can utilize the CCR1 receptor that is expressed on eosinophils. In addition, synthetic N-terminal variants of CC (e.g. Met-RANTES) and CXC (e.g. IL-8) chemokines function as receptor antagonists on eosinophils and neutrophils, whereas the native structures do not. Similarly, the CXC chemokine SDF-1.alpha. is a potent chemoattractant for leukocytes through activation of the receptor CXCR4/Fusin/LESTR, which is a fusion co-factor for the entry of HIV-1. CXCR4 mediated HIV-1 fusion can be inhibited in some cells by SDF-1.alpha.. Thus, despite the sequence similarities between certain chemokines of the same family, the binding and antagonist/agonist properties for HIV infection vary significantly.

Detailed Description Text (103):
Polypeptides of the invention are screened for receptor usage, inhibition of HIV infection, potency and breadth of activity against HIV infection, induction of calcium mobilization and angiogenesis. The assays are used to evaluate suppression of HIV-1 infection/replication in U87/CD4 cells (a human glioma cell line) expressing HIV-1 co-receptors and also in PBMCs.

Detailed Description Text (104):
The receptor-transfected U87/CD4 cells are obtainable by transfecting cells with an expression cassette encoding the respective receptors following standard protocols. The cells are maintained in Dulbecco's Minimal Essential Medium containing 10% fetal calf serum ("FCS"), glutamine, antibiotics, 1 .mu.g/ml puromycin (Sigma Chemicals) and 300 .mu.g/ml neomycin (G418; Sigma) and split twice a week. PBMCs are isolated from healthy blood donors by Ficoll-Hypaque centrifugation, then stimulated for 2-3 days with phytohemagglutinin ("PHA") (5 .mu.g/ml) and IL-2 (100 U/ml) (Simmons, et al., J. Virol. 70:8355-8360 (1996)). CD4.sup.+ T-cells are purified from the activated PBMCs by positive selection using anti-CD4 immunomagnetic beads (DYNAL Inc.), screened for CCR-5 defective alleles, and cells from allele defective or wild-type donors used depending on the assay. HIV isolates are obtainable from various sources including the NIAID HIV-1 Antigenic Variation study, or from similar programs organized by the US Department of Defense or the World Health Organization. Phenotypes of test viruses are tested by their ability to form syncytia ("SI") in MT-2 cells that are cultured in RPMI 1640 medium containing 10% FCS, glutamine and antibiotics, and split twice a week. Human CC-chemokines MIP-1.alpha., MIP-1.beta. and RANTES, and CXC-chemokines SDF-1.alpha. stocks are compared for purity and potency.

Detailed Description Text (105):
B. Assay for Inhibition of HIV Infection

Detailed Description Text (106):
Compounds of the present invention are tested against a panel of U87/CD4 cells stably expressing either CCR3, CCR5, CXC4 or CXCR4 receptors exposed to HIV-1/NSI strains SL-2 and SF162 (macrophage-tropic strains that utilize the RANTES, MIP-1.alpha. and MIP-1.beta. receptor CCR5 to gain entry into CD4.sup.+ cells) and the dual-tropic syncytium inducing strains 89.6 and 2028 (syncytium inducing dual tropic strains that can use CXCR4 and CCR3 in addition to CCR5 for entry). Lymphocytes and CD4.sup.+ T-cells from donors also are tested. Serial concentrations ranging from 0 to 500 nM of the cross-over proteins are used. RANTES, MPBA, MPBV and SDF-1.alpha. are used as controls. Inhibition of HIV infection is reported as a percentage of infection relative to modular protein and control concentrations.

Detailed Description Text (108):
Virus infectivity on the receptor expressing U87/CD4 cells is assessed by focus-

forming units (FFU) (Simmons, et al, Science 276:276-279(1997)). The FFU for viruses viruses using more than one co-receptor is assessed separately for each appropriate co-receptor expressing U87/CD4 cell type. Cells are seeded into 48 well trays at 1.times.104 cells/well overnight. The cells are then pre-treated for 30 minutes at 37.degree. C. with appropriate concentrations of chemokine in 75 .mu.l. 100 FFU of each virus in 75 .mu.l is added and incubated for 3 hours at 37.degree. C. Cells are are washed 3 times and 500 .mu.l of medium containing the appropriate chemokine at the correct concentration is added. After 5 days the cells are fixed for 10 minutes in cold acetone:methanol (1:1) and analyzed for p24 antigen production. Stand potency of cross-over chemokines against HIV infection

Detailed Description Text (109):
The breadth and potency of the inhibitory actions of the compounds of the present invention are tested against native CC-chemokines (MIP-1.alpha., MIP-1.beta. and RANTES) for M-tropic primary isolates of HIV-1, and against a native CXC-chemokine (SDF-1.alpha.) for T-tropic isolates in mitogen-stimulated primary CD4.sup.+ T-cells. The compounds are evaluated for their potency and spectrum of agonistic activity against HIV-1 strains relative to the native CC- and CXC-chemokines to identify the most active inhibitor of HIV-1 replication and the best template for therapeutic development. The properties and activities of M-Tropic and T-tropic primary HIV-1 isolates are recorded and compared to inhibition of infection by exposure to the cross-over chemokines relative to the HIV isolate designation, genetic subtype, and phenotype determined by ability of an isolate to form ("SI") or not form ("NSI") syncytia in MT-2 cells, the ability of an isolate to replicate efficiently in activated CD4.sup.+ T-cells from individuals homozygous for either wild-type or delta-32 CCR5 alleles, and the ability of an isolate to replicate in U87/CD4 cells stably expressing either CCR5 or CXCR4. The median ID.sub.50 and ID.sub.90 values (ng/ml) are calculated for each sample. A value of > indicates that 50% or 90% inhibition is not achieved at a chemokine concentration of the highest tested in any experiment. The means from two independent experiments are compared. FACS analysis of CCR5 and CXCR4 receptor expression levels, and/or competitive inhibition assay of the compounds of the present invention and receptor down-regulation also may be tested following standard protocols (Wu, et al., J. Exp. Med. 185:168-169(1997); and Trkola, et al., Nature 384:184-186(1996)).

Detailed Description Text (117):
AOP-RANTES Inhibits HIV-1 Replication In Vivo and In Vitro

Detailed Description Text (118):
Primary macrophage-tropic isolates of HIV-1 (R5 viruses) use CCR5 as a co-receptor for virus entry into target cells. CCR5 binds RANTES, MIP-1.alpha., and MIP-1.beta., and each of these chemokines can block virus entry into T cells at relatively high concentrations. N-terminal modifications of RANTES with higher affinity for CCR5 binding have been tested for inhibition of virus entry and replication in both T cells and macrophages in vitro. In addition, in vivo tests of the efficacy of these antagonists have been performed in SCID mice repopulated with human peripheral blood mononuclear cells (the "hu-PBL-SCID" model, described in Mosier, et al., Nature (London) 335:256-259 (1988) and Mosier, et al., Science 251:791-794 (1991)). AOP-RANTES (SEQ ID NO:2) is an effective inhibitor of M-tropic R5 virus replication in vitro at nanomolar concentration. The same compound substantially reduces virus levels when administered to hu-PBL-SCID mice just prior to virus infection and continuously for the ensuing week. Virus rebounds once treatment is stopped, but the recovered virus shows no mutations in the V3 region and has not altered co-receptor usage. First generation chemokine antagonists thus show antiviral efficacy in an animal model, and this antiviral effect may not select for viral variants with altered co-receptor utilization.

Detailed Description Text (119):
AOP-RANTES Inhibits HIV-1 Replication in hu-PBL-SCID Mice

Detailed Description Text (120):
Hu-PBL-SCID mice were generated by injection of 20.times.10.sup.6 PBMCs from an EBV-seronegative donor on day -14 or -15, using methods described in Mosier, Adv. Immunol. 63:79-125 (1996), Picchio, et al., J. Virol. 71:7124-7127 (1997), and Picchio, et al., J. Virol. 72:2002-2009 (1998). The mice were divided into groups of four mice each. Group 1 received implantation of an Alzet 2001 mini-osmotic pump (ALZA Pharmaceuticals, Palo Alto, Calif.) containing 2.5 mg/ml of AOP-RANTES (SEQ ID NO:2) (delivered at a rate of 1 .mu.l per hour for a minimum of 200 hours). Group 2 received implantation of a similar Alzet pump containing 2.5 mg/ml of BSA. The Alzet pumps were implanted subcutaneously on day -1 (14 days after the SCID mice were reconstituted). Thus, on day -1, delivery of AOP-RANTES began for mice in Group 1. On the following day (day 0), the Group 1 mice were given a single bolus injection of 1 mg AOP-RANTES and the Group 2 mice were given a single bolus injection of 1 mg BSA. One hour later, all mice were challenged with 1000 TCIDs of the M-tropic 242 HIV-1 isolate (Chesebro, et al., supra and Speck, et al., supra).

Detailed Description Text (121):
Virus infection was monitored by plasmid HIV RNA levels, which were determined using the Roche Amplicor HIV Monitor assay, a quantitative PCR determination with a limit of detection of 200 copies/ml. One AOP-RANTES mouse died on day 6 of a surgical complication of pump replacement. Plasma samples were obtained from all mice on day 7 (7 days after infection with HIV-1) and plasma HIV-1 RNA copy number measured on days 7 and 14 (after infection). Plasma RNA levels 200 are undetectable with the Roche assay, so two mice (#1 and #2) shown as 200 copies/ml on day 7 after infection had undetectable viral RNA levels. The reduction in plasma virus RNA levels by AOP-RANTES at day 7 after infection were highly significant ($p<0.001$).

Detailed Description Text (125):
AOP-RANTES has now been shown to be non-toxic and to significantly delay HIV-1 virus infection. Approximately 50 mg/kg of neutralizing antibody is required to block infection of the mice.

Detailed Description Text (127):
In Vitro Assays to Determine Inhibitory Effects of AOP-RANTES on Different HIV-1 Viruses

Detailed Description Text (128):
In vitro experiments have concentrated on the inhibitory effects of AOP-RANTES (SEQ ID NO:2) on dual-tropic viruses that can use other co-receptors in addition to CCR5. These in vitro experiments involved use of a p24 virus replication assay as described in Mosier, et al., supra. Briefly, culture media was sampled at day 7 post-infection with the relevant HIV-1 isolate, and p24 was measured in pg/ml via an an ELISA assay. The results are shown in FIG. 4, which shows that AOP-RANTES treatment in vitro inhibits R5 viruses but can enhance replication by R5.times.4 viruses in a CCR5-independent manner. Purified CD4 T cells were activated with PHA (and not IL-2) for 3 days prior to infection with HIV-1 242, 241 or the dual tropic 89.6 isolate. CD4 T cells were derived from either a normal donor (wild type "wt") or a donor homozygous for the 32 bp deletion in the CCR5 gene and thus having no expression of CCR5 on the cell surface ("del32") (as described in Picchio, et al., J. Virol. 71:7124-7127 (1997)).

Detailed Description Text (129):
As shown in FIG. 4, three different HIV-1 viruses were used: 241, 242, and 89.6. 89.6 is a dual-tropic HIV-1 isolate that uses multiple chemokine receptors, CCR2, CCR3, CCR5 and CXCR4, and is designated R2R3R5.times.4. Replication of HIV-1 242 in del32 CD4 T cells was enhanced by AOP-RANTES, but replication of HIV-1 89.6 in these T cells was unchanged by AOP-RANTES, as shown in FIG. 4.

Detailed Description Text (132):
Low Concentrations of AOP-RANTES Can Enhance Replication of Dual-Tropic HIV-1 in

PBMC Cultures from Selected Donors

Detailed Description Text (133):
Isolated PBMCs activated with PHA and IL-2 were infected with either HIV-1 242, 241 or 230 (see FIGS. 3 and 5) and viral replication monitored by p24 ELISA assay at days 4-18 of culture. Data in FIG. 5 are from day 11. Three independent PBMC donors (all CCR5 wt/wt) gave similar results as shown here, but other donors showed less enhancement of 241 replication, or even partial inhibition (See FIG. 4 where AOP-RANTES (SEQ ID NO:2) treatment of isolated CD4 T cells in vitro inhibits R5 viruses but can enhance R5.times.4 viruses in a CCR5-independent manner).

Detailed Description Text (135):
V3 Sequences of the Macrophage-tropic. CCR5 Using HIV-1 Molecular Clone Used in These Experiments and Virus Recovered from Hu-PBL-SCID Mice After AOP-RANTES Treatment

Detailed Description Text (136):
HIV-1 Clone 242 originated from B. Chesebro, and has the sequence indicated in FIG. 3 (SEQ ID NO:8). The 242 virus pool used in these experiments has a point mutation that resulted in a R to H change at position 21 (SEQ ID NO:9). Virus recovered from all AOP-RANTES-treated mice as well as control mice retained the 242/H V3 (SEQ ID NO:9) sequence.

Detailed Description Text (139):
N-nonanoyl-RANTES (2-68) Inhibits HIV-1 Replication in hu-PBL-SCID Mice

Detailed Description Text (140):
Fifteen SCID mice were reconstituted with 20.times.10.sup.6 peripheral blood mononuclear cells from an EBV-seronegative donor on day -14, using methods described in Mosier, Adv. Immunol. 63:79-125 (1996), Picchio, et al., J. Virol. 71:7124-7127 (1997), and Picchio, et al., J. Virol. 72:2002-2009 (1998). The mice were divided into three groups of five mice each. Group 1 received no additional treatment. Group 2 received implantation of an Alzet 2001 mini-osmotic pump containing 500 .mu.g of n-nonanoyl-RANTES (2-68) (SEQ ID NO:2) (delivered at a rate of 1 .mu.l per hour for a minimum of 200 hours). Group 3 received implantation of a similar Alzet pump containing 500 .mu.g of BSA. The Alzet pumps were implanted subcutaneously on day -1 (13 days after the SCID mice were reconstituted). Thus, on day -1, delivery of n-nonanoyl-RANTES (2-68) began for mice in Group 2. On day 0, all 15 mice were infected with 1000 TCIDs of HIV-1 242, an isolate described in Chesebro, et al., supra, and Speck, et al., supra. Mice in group 2 received an intraperitoneal injection of 1 mg n-nonanoyl-RANTES (2-68) one hour prior to virus injection. N-nonanoyl-RANTES (2-68), was suspended in saline solution and heated to 37.degree. C.

Detailed Description Text (141):
Virus infection was monitored by plasmid HIV RNA levels, which were determined using the Roche Amplicor HIV Monitor assay, a quantitative PCR determination with a limit of detection of 200 copies/ml. Plasma samples were obtained from all mice on day 7 (7 days after infection with HIV-1, 8 days after implantation of Alzet pumps in Groups 2 and 3), on day 14 (14 days after infection), and day 28 (28 days after infection). At the final time point, 3 of the 5 mice from each group were sacrificed for determination of the numbers of human CD3, CD4, CD8, and CD45-positive cells in two sites, the peritoneal cavity site of cell injection, and local lymph nodes ("LN") draining the peritoneal cavity. The frequency of human cell types was determined by staining with fluorochrome-labeled antibodies followed by fixation and flow cytometric analysis, as described in Picchio, et al., J. Virol. 72:2002-2009 (1998) and Picchio, et al., J. Virol. 71:7124-7127 (1997).

Detailed Description Text (142):
Results for HIV RNA levels (in copies/ml plasma) are generally shown in FIG. 6 as

log-transformed numbers. Log transformation is appropriate because viral replication replication is an exponential process, and small differences in viral load (e.g., <half log.sub.10) are insignificant. Cell recovery is expressed as a percentage of total recovered cells, and CD4 T cells are also expressed as a percentage of CD3 T cells, since CD4-positive cells are a subset of CD3-positive T cells. CD4 T cell recovery experiments illustrate that n-nonanoyl-RANTES (2-68) can block HIV-1 infection by a macrophage-tropic virus, 242 (now referred to as an R5 virus).

Detailed Description Text (143):
When virus RNA levels were undetectable, they are assigned the lowest value that could have been detected with the available volume of mouse plasma, e.g., 400 copies/ml. the number 400 in this context means 400 copies or fewer, or undetectable by the Roche Amplicor assay. Recovery of human cells at the end of the experiment has to be put into the context of what one would expect in the absence of HIV-1 infection. Historically, CD4 T cell counts account for 30-50% of human CD3 T cells in the peritoneal cavity and 40-70% of total T cells in lymph nodes. All mice in Group 2 (n-nonanoyl-RANTES (2-68) treated) had recovery of CD4-positive T cells in the range expected for uninfected hu-PBL-SCID mice. CD4 T cells accounted for 32-86% of total T cells in peritoneal cavity in the 3 Group 2 mice, and from 52-68% of T cells in LN. By contrast, CD4 T cells in control groups 1 and 3 accounted for 8-12% of total T cells in peritoneal cavity, showing the CD4 T cell depletion previously documented in HIV-1 infected mice. Only one mouse (#124 in group 3) shows lower than expected levels of human cell and HIV-1 viral RNA.

Detailed Description Text (148):
SCID mice were bred under specific pathogen flee conditions at Scripps Institute and tested for mouse IgM production at 8 weeks of age. Mice with <5 .mu.g/ml of IgM were engrafted with PBMCs prepared from EBV-seronegative donors from the Scripps General Clinical Research Center pool. SCID mice were injected with 20.times.106 PBMC intraperitoneally, and checked for plasma levels of human IgG after 12-13 days. Mice with >100 .mu.g/ml of human IgG were used for HIV-1 infection. Each experiment used mice generated from a single, different EBV-negative donor.

Detailed Description Text (149):
HIV-1 Virus Pools

Detailed Description Text (151):
Table 1 shows the V3 envelope sequences of HIV-1 242 recovered from hu-PBL-SCID mice treated with AOP- or NNY-RANTES (SEQ ID NO:2). Sequences in B (SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:12) were derived from the two mice in FIG. 8B. The R5.times.4 241 isolate has an E to Q change at position 24 (Chesebro, et al., supra) and retains the R at position 21 (SEQ ID NO:10). 242H would thus require 2 amino acid changes to change cell tropism and 242R only one amino acid change.

Detailed Description Text (153):
PBMC were collected from normal blood by density centrifugation. CD4.sup.+ T cells were separated by depletion of other cell types by antibody treatment and immunomagnetic bead separation. Whole PBMC or separated CD4.sup.+ T cells were cultured at 5.times.104 cells per well in 96 well microtiter plates. Cells were activated with PHA and IL-2 for 34 days, the medium replaced with concentrations of AOP- or NNY-RANTES (SEQ ID NO:2) ranging from 100 ng/ml to 1 pg/ml, cells incubated for 30 minutes at 37.degree. C., and then infected with 100 TCID of HIV-1 in the continued presence of modified RANTES. After overnight incubation, free virus was removed and fresh medium containing the original concentration of modified RANTES added. Culture medium was sampled on days 4, 7, and 10 after infection, and p24 HIV capsid antigen measured by ELISA.

Detailed Description Text (157):
Infection of hu-PBL-SCID mice with HIV-1 was determined by plasma HIV-1 RNA levels measured by the quantitative Roche PCR assay (Amplicor HIV Monitor, Roche Molecular

Systems, Somerville, N.J.). The limit of detection was 200-400 copies/ml depending on the plasma volume available. Depletion of CD4+T cells was measured by flow cytometry. Cells recovered from the peritoneal cavity or regional lymph nodes of hu-hu-PBL-SCID mice were stained with fluorescein- or phycoerythrin-labeled antibodies to human CD3, CD4, CD8, or CD45 and mouse H-2Kd (Pharmingen, San Diego, Calif.) and analyzed on a FACScan (Becton Dickinson, Mountain View, Calif.) flow cytometer. CD4+T cells are expressed as a percentage of total CD3+cells.

Detailed Description Text (161):
RNA was extracted from mouse plasma using the Qiagen viral RNA kit (Qiagen, Valencia, Calif.). RNA was convened to cDNA by reverse transcriptase-PCR (RT-PCR). cDNA was amplified by nested PCR using the following primers: outer V3 sense, CCAATTCCCATACATTATTG (SEQ ID NO:13); outer V3 anti-sense, ATTACAGTAGAAAAATTCCCC (SEQ ID NO:14); inner V3 sense, CAGTACAATGTACACATGGAATT (SEQ ID NO:15); inner V3 anti-sense, AATTTCTGGGTCCCCTCCTGA (SEQ ID NO:16). The final 356 bp product was cloned using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, Calif.), and the resulting product subject to automated sequencing (ABI, Perkins-Elmer, Foster City, Calif.). The final sequence encodes 54 amino acids 5' of V3 and 50 amino acids 3' of V3. Although only the translated V3 sequence is shown in Table 1, the entire sequence was examined and there were no mutations outside of V3.

Detailed Description Text (164):
The ability of AOP-RANTES and NNY-RANTES to inhibit R5 virus infection, including the R5 242 isolate of Chesebro, et al., supra, was confirmed by in vitro experiments (FIG. 7). The results show that NNY-RANTES (SEQ ID NO:2) and AOP-RANTES were both effective at inhibiting infection of activated PBMC with the SF162 as well as the two variants of the 242 HIV-1 isolate (see Table 1) and failed to inhibit infection with X4 isolates (data not shown). The CCR5 antagonists were able to reduce infection with the R5.times.4 241 virus (Chesebro, et al., supra) only at higher concentrations, and showed some enhancement of infection at lower concentrations (data not shown). NNY-RANTES was about three-fold more potent than AOP-RANTES at preventing infection with SF162, but was not more potent than AOP-RANTES at inhibiting either the 242H or 242R variants. HIV-1 242R (with a R rather than an H at position 21 of V3) was more resistant to inhibition than 242H with both CCR5 antagonists.

Detailed Description Text (166):
Three replicate experiments in hu-PBL-SCID mice were conducted to evaluate the in vivo efficacy of AOP- or NNY-RANTES (SEQ ID NO:2). Because of their rapid clearance from plasma, the CCR5 antagonists were administered at the rate of 2.5 .mu.g/hr by continuous infusion using subcutaneously implanted osmotic pumps. In addition, a single dose of 1 mg (.about.50 mg/kg) of each antagonist was injected just prior to virus infection. Serial plasma HIV RNA determinations were performed on the treated and control hu-PBL-SCID mice following infection with HIV-1 242. In the experiment shown in FIG. 8A, mice were infused with AOP-RANTES or BSA as a control. Two of the four mice treated with AOP-RANTES had undetectable viral RNA levels at the end of the 7 day infusion period, but virus levels increased in all mice once AOP-RANTES administration was halted. The one animal with viral RNA levels in the control range during treatment (AOP-R 4 in FIG. 8A) also had lower plasma levels of AOP-RANTES (See Table 2). AOP-RANTES was capable of reducing viral load but could not prevent HIV-1 infection despite plasma levels that were fully inhibitory in vitro (see FIG. 7). The inhibitory capacity of NNY-RANTES was tested in the next two experiments using BSA as a control. The results of the first experiment are shown in FIG. 8B. Four of 5 hu-PBL-SCID infused with NNY-RANTES had undetectable viral RNA levels at the end of the infusion period, and only 1 animal subsequently developed viremia (NNY-R 3 in FIG. 8B). NNY-RANTES treatment was thus successful in preventing R5 HIV-1 infection in 3 of 5 mice despite achieving lower plasma concentrations (Table 1) than AOP-RANTES. This experiment was repeated using a different human donor to generate hu-PBL-SCID mice. The results (FIG. 8C) were similar, with NNY-RANTES again preventing infection in 3 of 5 mice.

Detailed Description Text (167):
Table 2 sets forth data as to the recovery of CD4.sup.+ human T cells, antagonist levels, and plasma HIV RNA in hu-PBL-SCID mice treated with CCR5 antagonists. Steady-state plasma levels of N-modified RANTES in hu-PBL-SCID mice are shown to be 3.63.+-.0.8 ng/ml for AOP-RANTES and 0.76.+-.0.04 and 0.59.+-.0.16 ng/ml for NNY-RANTES.

Detailed Description Text (168):
The relative survival of human CD4.sup.+ T lymphocytes in hu-PBL-SCID mice treated with CCR5 antagonists was also measured. Both AOP- and NNY-RANTES were able to slow the depletion of CD4.sup.+ T cells, even in mice where HIV-1 infection was not prevented (See Table 2).

Detailed Description Text (170):
To determine if virus from hu-PBL-SCID mice that became infected despite treatment with AOP-or NNY-RANTES (SEQ ID NO:2) was evading the antagonists by mutating from CCR5 to CXCR4 co-receptor utilization, proviral DNA envelope genes were amplified and the region surrounding the V3 loop was sequenced, a critical determinant of co-receptor usage (Cocchi, et al., Nature Med. 2:1244-1247 (1996)). V3 sequences observed in the mice are shown in Table 1. In the first experiment (FIG. 8A), all mice treated with AOP-RANTES had the same sequence as the starting 242 virus isolate (which was found to contain an H in place of the published R at position 21, a change that had occurred prior to the initiation of these experiments). In the second experiment (FIG. 8B), HIV-1 recovered from the two mice that became infected despite treatment with NNY-RANTES differed in the V3 sequence. One mouse had the sequence of the starting 242 isolate (except for one clone with a replacement mutation at position 29), while the other mouse showed a reversion of the H at position 21 to the R present in the original molecular clone. The presence of H or R at position 21 in these isolates did not impact CCR5 usage but did impact susceptibility to NNY-RANTES (FIG. 7B). These results show that although sequence variation was occurring and there may have been selection for sequence variants that were less sensitive to NNY-RANTES inhibition, there was not rapid selection for HIV-1 variants that used alternative co-receptors for viral entry.

Detailed Description Text (171):
Example 19 establishes that NNY-RANTES is more effective than AOP-RANTES in preventing HIV-1 infection, and that neither antagonist selected for viruses capable capable of utilizing other co-receptors for virus entry. These results show that it is possible to block HIV-1 infection with N-terminally modified RANTES compounds in vivo. Inhibition of virus infection occurred with plasma levels of 0.4-0.9 ng/ml of NNY-RANTES and 4-5 ng/ml of AOP-RANTES during continuous administration of the antagonists, levels that are lower than the average concentration (.about.20 ng/ml) of native RANTES in human plasma (Weiss, et al., J. Infect. Dis. 176:1621-1624 (1997)). There has been one previous report of a chemokine receptor antagonist (AMD3100) that displayed efficacy against X4 HIV-1 infection in mice, albeit at higher concentrations (Datema, et al., Antimicrob. Agents Chemother. 40:750-754 (1996)), but this is the first report of antiviral activity of a CCR5 antagonist in vivo.

Detailed Description Text (172):
NNY-RANTES is as effective as a potent neutralizing antibody at preventing HIV-1 infection of hu-PBL-SCID mice (Parren, et al., AIDS 9:1-6 (1995) and Gauduin, et al., Nat. Med. 3:1389-1393 (1997)). Mice that were not protected from infection had lower vital RNA levels and higher CD4.sup.+ T cell counts than controls, suggesting that CCR5 antagonists may be useful in treating established infection.

Detailed Description Text (175):
The absence of co-receptor switch variants following either AOP- or NNY-RANTES administration is consistent with the slow rate of development of X4 viruses in

infected humans The absence of co-receptor switch variants following either AOP- or NNY-RANTES administration is consistent with the slow rate of development of X4 viruses in infected humans (Schuitemaker, et al., J. Virol. 65:356-363 (1991); Tersmette, et al., Lancet 1:983-985 (1989); and Connor, et al., J. Virol. 67:1772-1777 (1993)). Few mutations are required to change co-receptor usage (Speck, et al., al., J. Virol. 71:7136-7139 (1997)), suggesting that there must be significant biological barriers to switching from the R5 to the X4 virus phenotype. In addition, addition, therapies that target cellular rather than viral proteins are less likely to select for escape mutations. Therapies with multiple chemokine receptor antagonists may also reduce the chance for escape mutations. It appears that one virus (242R, FIG. 7) with reduced sensitivity to NNY-RANTES emerged during treatment, so mutations that alter sensitivity to CCR5 antagonists without changing co-receptor usage may be anticipated. Nonetheless, these would be less troubling than selection for more pathogenic X4 variants. The present in vivo results thus support the continuing development of co-receptor antagonists as viable candidates for the therapy of HIV-1 infection (Cairns, et al., Nature Med. 4:563-568 (1998)).

Detailed Description Paragraph Table (3):
TABLE 2 day 7 day 14 N-RANTES day 14 HIV RNA Exp. Mouse ng/ml % CD4.sup.+ T cells log.sub.10 copies/ml A AOP-R 1 4.51 17.1 3.98 AOP-R 2 4.77 29.2 6.07 AOP-R 3 4.03 27.7 3.86 AOP-R 4 1.24 14.3 6.72 mean .+-. SE 3.63 .+-. 0.8 22.1 .+-. 3.7 5.16 .+-. 0.73 BSA 1 <0.01 3.5 7.16 BSA 2 <0.01 11.9 6.06 BSA 3 <0.01 6.2 6.31 BSA 4 <0.01 4.4 6.12 mean .+-. SE -- 6.5 .+-. 1.9 6.41 .+-. 0.25 B NNY-R 1 0.82 86.1 <2.30.sup.a NNY-R 2 0.68 -- <2.30 NNY-R 3 0.86 32.7 4.72 NNY-R 4 0.70 -- <2.30 NNY-R 5 0.71 47.9 4.32 mean .+-. SE 0.76 .+-. 0.04 55.6 .+-. 15.9 -- BSA 1 <0.01 11.7 6.49 BSA 2 <0.01 12.3 6.36 BSA 3 <0.01 -- 5.12 BSA 4 <0.01 -- 6.34 mean .+-. SE -- 12.0 .+-. 0.3 6.08 .+-. 0.32 C NNY-R 1 0.49 nd.sup.b <2.30 NNY-R 2 0.41 nd <2.30 NNY-R 3 1.23 nd <2.30 NNY-R 4 0.38 nd 6.02 NNY-R 5 0.43 nd 5.59 mean .+-. SE 0.59 .+-. 0.16 -- -- BSA 1 <0.01 nd 6.49 BSA 2 <0.01 nd 6.36 BSA 3 <0.01 nd 5.12 BSA-4 <0.01 nd 4.56 BSA-5 <0.01 nd 5.21 mean .+-. SE -- -- 4.72 .+-. 0.36 .sup.a -- indicates that the data was below the limit of detection of 200 copies/ml .sup.b -- not done

Other Reference Publication (2):
Alkhatib, Ghalib, et al., "CC CKR5: A RANTES, MIP-1.alpha., MIP-1.beta. Receptor as a Fusion Cofactor for Macrophage-Tropic HIV-1", Science, vol. 272, pp. 1955-1958 (1996).

Other Reference Publication (4):
Arenzana-Seisdedos, Fernando, et al., "HIV Blocked by Chemokine Antagonist", Nature, Nature, vol. 383, p. 400 (1996).

Other Reference Publication (5):
Berger, et al., "A New Classification for HIV-1", Nature, vol. 391, p. 240 (1998).

Other Reference Publication (6):
Cairns, et al., "Chemokines and HIV-1 Second Receptors: The Therapeutic Connection", Connection", Nature Med., vol. 4, No. 5, pp. 563-568 (1998).

Other Reference Publication (9):
Choe, et al., "The .beta.-Chemokine Receptors CCR3 and CCR5 Facilitate Infection by Primary HIV-1 Isolates", Cell, vol. 85, pp. 1135-1148 (1996).

Other Reference Publication (10):
Cocchi, et al., "Identification of RANTES, MIP-1.alpha., and MIP-.beta. as the Major HIV-Suppressive Factors Produced by CD8+ T Cells", Science, vol. 270, pp. 1811-1815 (1995).

Other Reference Publication (11):
Cocchi, et al., "The V3 Domain of the HIV-1 gp 120 Envelope Glycoprotein is Critical

Critical for Chemokine-Mediated Blockade of Infection", Nature Med., vol. 2, No. 11, 11, pp. 1244-1247 (1996).

Other Reference Publication (16):
Deng, et al., "Identification of a Major Co-Receptor for Primary Isolates of HIV-1", Nature, vol. 381, pp. 661-666 (1996).

Other Reference Publication (17):
Doranz, et al., "A Dual-Tropic Primary HIV-1 Isolate That Uses Fusin and the .beta.-the .beta.-Chemokine Receptors CKR-5, CKR-3, and CKR-2B as Fusion Cofactors", Cell, vol. 85, pp. 1149-1158 (1996).

Other Reference Publication (20):
Gauduin, et al., "Passive Immunization With a Human Monoclonal Antibody Protects hu-PBL-SCID Mice Against Challenge by Primary Isolates of HIV-1", Nat.Med., vol. 3, No. 12, pp. 1389-1393 (1997).

Other Reference Publication (23):
Mack, et al., "Aminooxypentane-RANTES Induces CCR5 Internalization but Inhibits Recycling: A Novel Inhibitory Mechanism of HIV Infectivity", J.Exp.Med., vol. 187, No. 8, pp. 1215-1224 (1998).

Other Reference Publication (26):
Mosier, et al, "Rapid Loss of CD4+ T Cells in Human-PBL-SCID Mice by Noncytopathic HIV Isolates", Science, vol. 260, pp. 689-692 (1993).

Other Reference Publication (28):
McKnight, et al., "HIV-2 and SIV Infection of Nonprimate Cell Lines Expressing Human Human CD4: Restrictions to Replication at Distinct Stages", Virology, vol. 201, pp. 8-18 (1994).

Other Reference Publication (31):
Parren, et al., "Protection Against HIV-1 Infection in hu-PBL-SCID Mice by Passive Immunization With a Neutralizing Human Monoclonal Antibody Against the gp120 CD4-Binding Site", AIDS, vol. 9, No. 6, pp. 1-6 (1995).

Other Reference Publication (32):
Paxton, et al., "Reduced HIV-1 Infectability of CD4+ Lymphocytes From Exposed-Uninfected Individuals: Association With Low Expression of CCR5 and High Production of .beta.-Chemokines", Virology, vol. 244, pp. 66-73 (1998).

Other Reference Publication (38):
Schuitemaker, et al., "Monocytotropic Human Immunodeficiency Virus Type 1 (HIV-1) Variants Detectable in all Stages of HIV-1 Infection Lack T-Cell Line Tropism and Syncytium-Inducing Ability in Primary T-Cell Culture", J.Virol., vol. 65, No. 1, pp. 356-363 (1991).

Other Reference Publication (39):
Simmons, et al., "Potent Inhibition of HIV-1 Infectivity in Macrophages and Lymphocytes by a Novel CCR5 Antagonist", Science, vol. 276, pp. 276-279 (1997).

Other Reference Publication (43):
Trkola, et al., "CD4-Dependent, Antibody-Sensitive Interactions Between HIV-1 and its Coreceptor CCR-5", Nature, vol. 384, pp. 184-187 (1996).

Other Reference Publication (47):
Wu, et al., "CCR5 Levels and Expression Pattern Correlate with Infectability by Macrophage-Tropic HIV-1 In Vitro", J.Exp.Med., vol. 185, No. 9, pp. 1681-1691 (1997).

☐ 8. Document ID: US 5908845 A

L3: Entry 8 of 13 File: USPT Jun 1, 1999

DOCUMENT-IDENTIFIER: US 5908845 A
TITLE: Polyether nucleic acids

Brief Summary Text (3):
An antisense oligonucleotide (e.g., antisense oligodeoxyribonucleotide) may bind its its target nucleic acid either by Watson-Crick base pairing or Hoogsteen and anti-Hoogsteen base pairing. To this effect see, Thuong and Helene (1993) Sequence specific recognition and modification of double helical DNA by oligonucleotides Angev. Chem. Int. Ed. Engl. 32:666. According to the Watson-Crick base pairing, heterocyclic bases of the antisense oligonucleotide form hydrogen bonds with the heterocyclic bases of target single-stranded nucleic acids (RNA or single-stranded DNA), whereas according to the Hoogsteen base pairing, the heterocyclic bases of the target nucleic acid are double-stranded DNA, wherein a third strand is accommodated in the major groove of the B-form DNA duplex by Hoogsteen and anti-Hoogsteen base pairing to form a triplex structure.

Brief Summary Text (4):
According to both the Watson-Crick and the Hoogsteen base pairing models, antisense oligonucleotides have the potential to regulate gene expression and to disrupt the essential functions of the nucleic acids. Therefore, antisense oligonucleotides have possible uses in modulating a wide range of diseases.

Brief Summary Text (7):
Gene expression involves few distinct and well regulated steps. The first major step of gene expression involves transcription of a messenger RNA (mRNA) which is an RNA sequence complementary to the antisense (i.e., -) DNA strand, or, in other words, identical in sequence to the DNA sense (i.e., +) strand, composing the gene. In eukaryotes, transcription occurs in the cell nucleus.

Brief Summary Text (10):
There are also evidence that in some cases gene expression is downregulated by endogenous antisesnse RNA repressors that bind a complementary mRNA transcript and thereby prevent its translation into a functional protein. To this effect see Green et al. (1986) The role of antisense RNA in gene regulation. Ann. Rev. Biochem. 55:569.

Brief Summary Text (17):
At the transcription level, antisense or sense oligonucleotides or analogs that bind bind to the genomic DNA by strand displacement or the formation of a triple helix, may prevent transcription. To this effect see, Thuong and Helene (1993) Sequence specific recognition and modification of double helical DNA by oligonucleotides Angev. Chem. Int. Ed. Engl. 32:666.

Brief Summary Text (18):
At the transcript level, antisense oligonucleotides or analogs that bind target mRNA mRNA molecules lead to the enzymatic cleavage of the hybrid by intracellular RNase H. To this effect see Dash et al. (1987) Proc. Natl. Acad. Sci. U.S.A., 84:7896. In this case, by hybridizing to the targeted mRNA, the oligonucleotides or oligonucleotide analogs provide a duplex hybrid recognized and destroyed by the

RNase H enzyme. Alternatively, such hybrid formation may lead to interference with correct splicing. To this effect see Chiang et al. (1991) Antisense oligonucleotides oligonucleotides inhibit intercellular adhesion molecule 1 expression by two distinct mechanisms. J. Biol. Chem. 266:18162. As a result, in both cases, the number of the target mRNA intact transcripts ready for translation is reduced or eliminated.

Brief Summary Text (19):
At the translation level, antisense oligonucleotides or analogs that bind target mRNA molecules prevent, by steric hindrance, binding of essential translation factors (ribosomes), to the target mRNA, as described by Paterson et al. (1977) Proc. Natl. Acad. Sci. U.S.A., 74:4370, a phenomenon known in the art as hybridization arrest, disabling the translation of such mRNAs.

Brief Summary Text (20):
Thus, antisense sequences, which as described hereinabove may arrest the expression of any endogenous and/or exogenous gene depending on their specific sequence, attracted much attention by scientists and pharmacologists who were devoted at developing the antisense approach into a new pharmacological tool. To this effect see Cohen (1992) Oligonucleotide therapeutics. Trends in Biotechnology, 10:87.

Brief Summary Text (21):
For example, several antisense oligonucleotides have been shown to arrest hematopoietic cell proliferation (Szczylik et al (1991) Selective inhibition of leukemia cell proliferation by BCR-ABL antisense oligodeoxynucleotides. Science 253:562), growth (Calabretta et al. (1991) Normal and leukemic hematopoietic cell manifest differential sensitivity to inhibitory effects of c-myc antisense oligodeoxynucleotides: an in vitro study relevant to bone marrow purging. Proc. Natl. Acad. Sci. U.S.A. 88:2351), entry into the S phase of the cell cycle (Heikhila et al. (1987) A c-myc antisense oligodeoxynucleotide inhibits entry into S S phase but not progress from G(0) to G(1). Nature, 328:445), reduced survival (Reed et al. (1990) Antisense mediated inhibition of BCL2 prooncogene expression and and leukemic cell growth and survival: comparison of phosphodiester and phosphorothioate oligodeoxynucleotides. Cancer Res. 50:6565) and prevent receptor mediated responses (Burch and Mahan (1991) Oligodeoxynucleotides antisense to the interleukin I receptor m RNA block the effects of interleukin I in cultured murine and human fibroblasts and in mice. J. Clin. Invest. 88:1190). For use of antisense oligonucleotides as antiviral agents the reader is referred to Agrawal (1992) Antisense oligonucleotides as antiviral agents. TIBTECH 10:152.

Brief Summary Text (22):
For efficient in vivo inhibition of gene expression using antisense oligonucleotides or analogs, the oligonucleotides or analogs must fulfill the following requirements (i) sufficient specificity in binding to the target sequence; (ii) solubility in water; (iii) stability against intra- and extracellular nucleases; (iv) capability of penetration through the cell membrane; and (v) when used to treat an organism, low toxicity.

Brief Summary Text (23):
Unmodified oligonucleotides are impractical for use as antisense sequences since they have short in vivo half-lives, during which they are degraded rapidly by nucleases. Furthermore, they are difficult to prepare in more than milligram quantities. In addition, such oligonucleotides are poor cell membrane penetraters, see, Uhlmann et al. (1990) Chem. Rev. 90:544.

Brief Summary Text (27):
Oligonucleotides can be modified either in the base, the sugar or the phosphate moiety. These modifications include the use of methylphosphonates, monothiophosphates, dithiophosphates, phosphoramidates, phosphate esters, bridged phosphorothioates, bridged phosphoramidates, bridged methylenephosphonates,

dephospho internucleotide analogs with siloxane bridges, carbonate bridges, carboxymethyl ester bridges, carbonate bridges, carboxymethyl ester bridges, acetamide bridges, carbamate bridges, thioether bridges, sulfoxy bridges, sulfono bridges, various "plastic" DNAs, x-anomeric bridges and borane derivatives. For further details the reader is referred to Cook (1991) Medicinal chemistry of antisense oligonucleotides--future opportunities. Anti-Cancer Drug Design 6:585.

Brief Summary Text (39):
There is thus a widely recognized need for, and it would be highly advantageous to have, oligonucleotide analogs devoid of these drawbacks which are characterized by (i) sufficient specificity in binding to target sequences; (ii) solubility in water; (iii) stability against intra- and extracellular nucleases; (iv) capability of penetrating through cell membranes; and (v) when used to treat an organism, low toxicity, properties that collectively render an oligonucleotide analog highly suitable as an antisense therapeutic drug.

Brief Summary Text (71):
The present invention successfully addresses the shortcomings of the presently known configurations by providing an oligonucleotide analog characterized by (i) sufficient specificity in binding its target sequence; (ii) solubility in water; (iii) stability against intra- and extracellular nucleases; (iv) capability of penetrating through the cell membrane; and (v) when used to treat an organism, low toxicity, properties collectively rendering the oligonucleotide analog of the present invention highly suitable as an antisense therapeutic drug.

Detailed Description Text (2):
The present invention is a compounds that are not polynucleotides yet which bind to complementary DNA and RNA sequences, the compounds according to the invention include naturally occurring necleobases or other nucleobases binding moieties (also referred herein as nucleobase analogs) covalently bound to a polyether backbone, which can be used as oligonucleotide analogs in for example antisense procedures. The oligonucleotide analogs according to the present invention include a new acyclic biopolymer backbone which best fulfills the five criteria for selecting antisense oligonucleotide analogs listed in the background section above.

Detailed Description Text (3):
The synthesis, structure and mode of operation of antisense oligonucleotide analogs according to the present invention may be better understood with reference to the drawings and accompanying descriptions.

Detailed Description Text (40):
The present invention is further directed at therapeutic and/or prophylactic uses for polyether nucleic acids (ENAs). Likely therapeutic and prophylactic targets according to the invention include but are not limited to human papillomavirus (HPV), herpes simplex virus (HSV), candidia albicans, influenza virus, human immunodeficiency virus (HIV), intracellular adhesion molecules (ICAM), cytomegalovirus (CMV), phospholipase A2 (PLA2), 5-lipoxygenase (5-LO), protein kinase C (PKC), and RAS oncogene.

Detailed Description Text (53):
Second, one of the major drawbacks of PNAs when used as antisense molecules is that PNA-DNA hybrids are characterized by high melting temperature (Tm). For example, the Tm value for a duplex such as PNA-T.sub.10 -dA.sub.10 is greater than 70.degree. C., whereas the Tm value of the equivalent native double stranded DNA (dT.sub.10 -dA.sub.10) is nearly three fold lower, about 24.degree. C. Because PNAs bind complementary sequences so strongly, at body temperature (e.g., 37.degree. C.) PNAs lack the specificity to their intended counterparts and end up binding not just to target sequences but also to other strands of DNA, RNA, or even proteins, incapacitating the cell in unforeseen ways. PNAs act as a micelle when the lysine residues are solvated. PNAs are poorly miscible in water, while the hydrophobic

nature of the backbone have a tendency to seek for a nonpolar environment e.g., the bases of the natural complementary DNA. These hydrophobic interactions are the major major driving force for the formation of highly stable PNA-DNA hybrid and therefore very high Tm values for such hybrids. The unique solubility nature of ENAs, by conserving the hydrophobic-hydrophilic properties of polyethers such as PEG, yield Tm values slightly higher than natural DNA, yet much lower values than PNAs, which moderate values are of great importance for specificity.

Detailed Description Text (55):
Fourth, PEG is approved by the FDA for parenteral use, topical application, and as a a constituent of suppositories, nasal sprays, foods and cosmetics. PEG is of low toxicity when administered orally or parenterally, and only large quantifies involve adverse reactions. See, Smyth, H. F. et al. (1955) J. Am. Pharm. Assoc., 34:27. Evidences accumulated experiencing administration of PEG-protein conjugates, suggest that both the plasma half-lives (circulating time) of PEG conjugated proteins and their bioavailability improves as compared with the native proteins, which improvement is accompanied by improved efficacy. Ganser et al. (1989) Blood, 73:31, observed less side effects at lower dosage using PEG-modifications. Reduced toxicity has been observed with several PEG-modified enzymes, see Fuertges et al. (1990) J. Contr. Release, 11:139. Another advantage in exploiting the improved pharmacokinetics of PEG is the option of administrating bolus injections instead of continuous intravenous infusions, as described by Pizzo (1991) Adv. Drug Del. Rev. 6:153. In the preferred embodiments of the invention, ENAs include a PEG backbone and/or are conjugated to PEG exoconjugates and therefore enjoy the above listed advantages.

Detailed Description Text (100):
The dried polymer is then condensed with a second compound following formula V to which a second base (B.sub.2) is attached (e.g., compound H-B.sub.2) in dry DMF in a manner as described above under condensation. Such cycles are repeated as much as needed to form appropriate antisense sequence, wherein in each tri-stages cycle one additional monomer is sequentially added to the growing chain.

Detailed Description Text (103):
The polymeric support to which the antisense sequence is attached, is treated with concentrated ammonium hydroxide for 16 hours at 55.degree. C. The polymeric support is washed with water, methanol and with ether.

Other Reference Publication (1):
Burch et al, "Oligonucleotides Antisense to the Interlukin 1 Receptor mRNA Block the the Effects of Interlukin 1 in Cultures Murine and Human Fibroblasts and in Mice", J. Clin. Inv., vol. 88, pp. 1190-1196, (1991).

Other Reference Publication (2):
Calabretta et al, "Normal and leukemic hematopoietic cells manifest differential sensitivity to inhibitory effects of c-myb antisense ologideoxynucleotides: An in vitro study relevant to bone marrow purging", Proc. Natl. Acad. Sci. USA, vol. 88, pp. 2351-2355, (1991).

Other Reference Publication (4):
Heikkila et al, "A c-myb antisense ologideoxynucleotide inhibits entry into S phase but not progress from G.sub.0 to G.sub.1 ", Nature, vol. 328, 445-449, (1987).

Other Reference Publication (8):
Szczylik et al, "Selective Inhibition of Leukemia Cell Proliferation by BCR-ABL Antisense Ologideoxynucleotides", Science, vol. 253, pp. 562-565, (1991).

Other Reference Publication (9):
Uhlmann et al, "Antisense Ologideoxynucleotides: A New Therepeutic Principle", Chem. Chem. Rev., vol. 90, No. 4, pp. 544-584, (1990).

Other Reference Publication (17):
Agrawal, S., "Antisense ologideoxynucleotides as antiviral agents", Tibtech, vol. 10, No. 5, pp. 152-158, (1992).

Other Reference Publication (18):
Wahlestedt, C., "Antisense ologideoxynucleotide strategies in neuropharmacology", TIPS, vol. 15, pp. 42-46, (1994).

Other Reference Publication (19):
Cook, P.D., "Medicinal chemistry of antisense ologideoxynucleotides -- future opportunities", Anti-Cancer Drug design, vol. 6, pp. 585-607, (1991).

Other Reference Publication (20):
Green, et al, "The Role of Antisense RNA in Gene regulation", Ann. Rev. Biochem., vol. 55, pp. 569-597, (1986).

☐ 9. Document ID: US 5707648 A

L3: Entry 9 of 13 File: USPT Jan 13, 1998

DOCUMENT-IDENTIFIER: US 5707648 A
**** See image for Certificate of Correction ****
TITLE: Transparent liquid for encapsulated drug delivery

Detailed Description Text (41):
Peptidyl proteinase inhibitors are another category of peptides and peptide analogues that may be usefully incorporated into the drug composition. Particularly preferred are: inhibitors of metalloproteinases, such as collagenase and elastase, which are useful in treating certain metastatic cancers and certain inflammatory diseases, such as arthritis; inhibitors of proteases coded on viral genomes, such as the HIV-1 and HIV-2 viral proteases; inhibitors of angiotensin converting enzyme (ACE inhibitors) or of renin, useful in the treatment of hypertension; and inhibitors of blood clotting cascades proteases, such as thrombin inhibitors, useful for treating thrombosis. Also useful as antithromobics are peptides and polypeptide fragments of the leech protein hirudin, as well as analogues of these fragments and hirudin itself.

Detailed Description Text (50):
Also, suitable agents include nucleosides, nucleotides and their polymers. Suitable nucleosides include 3'-azido-3'-deoxythimidine, 2',3'-dideoxy-derivatives of adenosine, cytidine, inosine, thymidine or guanosine. Suitable polynucleotides include "anti-sense" nucleotides having 3 to 30 nucleotide bases with nucleotide sequences complimentary to those coding for viral proteins or RNA's, oncogene proteins or RNA's, or inflammatory proteins or RNA's. Also useful are polynucleotides having 3 to 30 bases capable of forming triple helix structures with the DNA coding for the above.

Detailed Description Text (61):
The delivery composition of the present invention can be formulated with a high melting oil, that is, an oil with a melting point above room temperature (22.degree.-23.degree. C.), preferably above about 30.degree. C., in order to formulate a composition which is a solid at room temperature. Also, high melting

surfactants such as a C.sub.10-40 ester of a long chain fatty acid and alcohols having at least about 12 carbon atoms, wherein these surfactants have melting points points above room temperature, preferably above about 30.degree. C. Preferably, the composition will melt at body temperatures, generally between about 35.degree.-40.degree. C. The amount of high melting oil and the melting point of that oil can vary, but the final composition is solid at room temperatures. The solid composition composition can be used as a suppository transport vehicle or as an oral transport vehicle. The composition can either be formulated directly with the high melting oil, or the composition can be formulated first, after which the high melting oil is blended with the composition. Such high melting oils are well known in the art and include, for example, partially hydrogenated coconut oils, palm oils, cocobutter, hydrogenated peanut oil, and various hydrogenated vegetable oils, along with combinations thereof. Preferred oils include hydrogenated coconut and palm oils and mixtures thereof. The high molecular weight PEG components can also be used to formulate a solid composition.

☐ 10. Document ID: US 5693607 A

L3: Entry 10 of 13 File: USPT Dec 2, 1997

DOCUMENT-IDENTIFIER: US 5693607 A
TITLE: Uses of TGF-.beta. receptor fragment as a therapeutic agent

Drawing Description Text (23):
The s.beta.-RII fragments of the present invention may be used to treat viral infections in which there is an overproduction of TGF-.beta. and immunosuppression. Examples of viruses with which TGF-.beta. excess is associated include, but are not limited to, hepatitis C, lymphocytic choriomeningitis, human immunodeficiency virus (HIV), and human T cell lymphotropic virus (HTLV-1), the latter being discussed in Kim et al. ((1991) Mol. Cell. Biol. 11:5222-28).

Drawing Description Text (27):
The administration of s.beta.-RII fragments of the present invention may be used in fibroproliferative disorders. As mentioned above, animal models of glomerulonephritis have shown good results with anti-TGF-.beta. antibodies blocking excess TGF-.beta.. These antibodies will be difficult to deliver because they have a high molecular weight and they may result in severe allergic reactions when they are derived from other species. Thus, it would be preferable to administer a lower molecular weight, native protein or close analog, such as s.beta.-RII, in glomerulonephritis. Kidney diseases associated with TGF-.beta. excess include, but are not limited to, mesangial proliferative glomerulonephritis, crescentic glomerulonephritis, diabetic nephropathy, renal interstitial fibrosis, renal fibrosis in transplant patients receiving cyclosporin, and HIV-associated nephropathy. These conditions are associated with excessive fibrous tissue formation which administration of s.beta.-RII should suppress.

Drawing Description Text (47):
Depending on the mode of administration, the s.beta.-RII composition may be in the form of liquid or semi-solid dosage preparations, such as for example, liquids, suspensions or the like. Alternatively, a solution of s.beta.-RII may be placed into an implant, such as an osmotic pump, for the slow release of s.beta.-RII over an extended period of time. Alternatively, s.beta.-RII may be provided in sustained release carrier formulations such as semi-permeable polymer carriers in the form of

suppositories or microcapsules. See, for instance, U.S. Pat. No. 3,773,919 for Microcapsular Sustained Release Matrices Including Polylactides; Sidmon et al., Biopolymers 22 (1), 547-556 (1983) for copolymers of L-glutamic acid and .gamma.-ethyl-L-glutamate; Langer et al., J. Biomed. Res. 15, 167-277 (1981) for poly(2-hydroxyethylmethacrylate) or the like. Finally, receptor fragmentation and modifications, such as fusion of the s.beta.-RII fragment with human immunoglobulin (IgG) or with polyethylene glycol (PEG) so as to extend the half life of the s.beta.-RII fragment, are other alternative forms of administration.

Detailed Description Text (27):
An oligonucleotide was synthesized with the antisense sequence of the TGF-.beta. type II receptor from nucleotides 553-583 (nucleotides are numbered according to Lin et al., 1992) with the exception of the codon for Asn.sup.106 which was changed such that a stop codon would be inserted. The sequence of the oligonucleotide used to create this mutation is 5'-TAGCAACAAGTCAGGTTAGCTGGTGTTATATTC-3' (SEQ ID NO:7). This primer in combination with the UssDNA described above was used to carry out an in vitro mutagenesis experiment (Kunkel et al., (1985) Proc. Natl. Acad. Sci. USA 82:488-492). Clones containing the desired mutation were identified by nucleotide sequencing. A clone containing a stop codon in place of Asn.sup.106 was named BS/.beta.RIIs.

Other Reference Publication (45):
Lazdins, J.K., et al., "TGF-.beta.: Upregulator of HIV Replication in Macrophages", Res. Virol. (1991) 142:239-242.

☐ 11. Document ID: US 5677439 A

L3: Entry 11 of 13 File: USPT Oct 14, 1997

DOCUMENT-IDENTIFIER: US 5677439 A
TITLE: Oligonucleotide analogues containing phosphate diester linkage substitutes, compositions thereof, and precursor dinucleotide analogues

Brief Summary Text (4):
An antisense compound is a compound that binds to or hybridizes with a nucleotide sequence in a nucleic acid, RNA or DNA, to inhibit the function or synthesis of said nucleic acid. Because of their ability to hybridize with both RNA and DNA, antisense compounds can interfere with gene expression at the level of transcription, RNA processing or translation.

Brief Summary Text (5):
Antisense molecules can be designed and synthesized to prevent the transcription of specific genes to mRNA by hybridizing with genomic DNA and directly or indirectly inhibiting the action of RNA polymerase. An advantage of targeting DNA is that only small amounts of antisense compounds are needed to achieve a therapeutic effect. Alternatively, antisense compounds can be designed and synthesized to hybridize with with RNA to inhibit post-transcriptional modification (RNA processing) or protein synthesis (translation) mechanisms. Exemplary target RNAs are messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA) and the like. Examples of processing and translation mechanisms include splicing of pre-mRNA to remove introns, capping of the 5' terminus of mRNA, hybridization arrest and nuclease mediated mRNA hydrolysis.

Brief Summary Text (6):
At the present time, however, the development of practical scientific and therapeutic applications of antisense technologies is hampered by a number of technical problems. Klausher, A., Biotechnology, 8:303-304 (1990). Synthetic antisense molecules are susceptible to rapid degradation by nucleases that exist in target cells. The oligonucleoside sequences of antisense DNA or RNA, for example, are destroyed by exonucleases acting at either the 5' or 3' terminus of the nucleic acid. In addition, endonucleases can cleave the DNA or RNA at internal phosphodiester linkages between individual nucleosides. As a result of such cleavage, the effective half-life of administered antisense compounds is very short, necessitating the use of large, frequently administered, dosages.

Brief Summary Text (7):
Another problem is the extremely high cost of producing antisense DNA or RNA using available semiautomatic DNA synthesizers. It has recently been estimated that the cost of producing one gram of antisense DNA is about $100,000. Armstrong, L., Business Week, Mar. 5, 1990, page 89.

Brief Summary Text (8):
A further problem relates to the delivery of antisense agents to desired targets within the body and cell. Antisense agents targeted to genomic DNA must gain access to the nucleus (i.e. the agents must permeate the plasma and nuclear membrane). The need for increased membrane permeability (increased hydrophobicity) must be balanced, however, against the need for aqueous solubility (increased hydrophilicity) in body fluid compartments such as the plasma and cell cytosol.

Brief Summary Text (9):
A still further problem relates to the stability of antisense agents whether free within the body or hybridized to target nucleic acids. Oligonucleotide sequences such as antisense DNA are susceptible to steric reconfiguration around chiral phosphorous centers.

Brief Summary Text (10):
Gene targeting via antisense agents is the inevitable next step in human therapeutics. Armstrong, supra at 88. The successful application of antisense technology to the treatment of disease however, requires finding solutions to the problems set forth above.

Brief Summary Text (11):
One approach to preparing antisense compounds that are stable, nuclease resistant, inexpensive to produce and which can be delivered to and hybridize with nucleic acid targets throughout the body is to synthesize oligonucleoside sequences with modifications in the normal phosphate-sugar backbone structure.

Brief Summary Text (14):
Phosphorothioate modified phosphodiester linkages refer to phosphodiester bonds in which one or more of the bridging oxygen atoms is replaced by sulfur. Such linkages, however, are not suitable for use in antisense compounds. The retention of the chiral phosphorus center results in steric variation of monothioates. Further, both mono- and dithioates lack sequence specific hybridization and both are rapidly cleared from the plasma. The high affinity of phosphorothioates for glass and plastic also makes synthesis of these compounds difficult and inefficient.

Brief Summary Text (26):
Although the carbonate, carbamate and silyl linked oligonucleoside sequences have the requisite nuclease resistance to make them attractive candidates as antisense reagents, their ability to function in this capacity has not yet been reported. Further, the ability of these oligomers to be taken up by cells in culture has not been reported. A potential drawback with these oligomers is their reported low

solubility in aqueous solution. It is not clear whether sufficient concentrations can be obtained for their effective use in biological experiments, although solubility could presumably be increased by introduction of hydrophilic groups into the molecules.

Detailed Description Text (41):
In a preferred embodiment, the antisense compounds have polyethyalkyleneglycol at both the 5' and 3' termini and have the formula: ##STR13## where R is OH, SH, NR.sup.2 R.sup.3 wherein R.sup.2 and R.sup.3 are independently hydrogen or C.sub.1 -C.sub.6 alkyl, or NHR.sup.4 wherein R.sup.4 is C.sub.1 -C.sub.12 acyl;

Detailed Description Text (52):
The compounds of the present invention are useful as antisense agents. Antisense agents hybridize with a complementary nucleotide sequence in a target nucleic acid to inhibit the translational or transcriptional function of said target nucleic acid. The target nucleic acid may be either RNA or DNA.

Detailed Description Text (53):
Antisense compounds of the present invention comprise oligonucleoside sequences of from about 6 to about 200 bases having homopolymer or heteropolymer sequences comprising bases selected from the group consisting of adenine (A), cytosine (C), guanine (G) uracil (U), thymine (T) and modifications of these bases. Particular sequences are selected on the basis of their desired target. The sequence selected hybridizes with the target nucleic acid. Exemplary targets include the MYC oncogene, the RAS oncogene, and vital nucleic acids.

Detailed Description Text (89):
Those skilled in the art will appreciate that other means of synthesizing oligonucleotides can be modified in an analogous manner to produce diol-terminated antisense oligonucleotides.

Detailed Description Text (90):
The compounds of the present invention are useful in treating mammals with hereditary disorders or diseases associated with altered genetic expression mechanisms. At present, attempts are underway to develop antisense therapies for use in treating vital infections such as HIV, cytomegalovirus, herpes simplex, hepatitis B, papilloma virus and picorna virus; cancers of the lung, colon, cervix, breast and ovary; inflammatory diseases; and diseases of the immune system such as acquired immunodeficiency syndrome (AIDS), hematological neoplasma and hyperproliferative disorders. Armstrong, supra at 89; Klausher, supra at 303, 304.

Detailed Description Text (104):
Compositions for rectal administrations are preferably suppositories which can be prepared by mixing the compounds of the present invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethyleneglycol or a suppository wax, which are solid at ordinary temperatures but liquid at body temperature and therefore, melt in the rectum or vaginal cavity and release the active component.

Detailed Description Text (291):
Ability of TEG-Antisense Oligomers to Inhibit Protein Expression and Growth in Human Human Tumor Cell Lines and PHA Stimulation of Peripheral Blood Lymphocytes

Detailed Description Text (292):
It has been demonstrated by others (Heikkila, R. et al., Nature, 328:445-449, 1987) that unmodified antisense oligonucleotides directed towards the initiation codon region of the c-myc oncogene could inhibit the expression of c-myc protein in PHA stimulated peripheral blood lymphocytes (PBL) resulting in a block in the progression of cells into the S-phase of the cell cycle. C-myc directed antisense DNA was also shown to inhibit the growth of HL-60 human erytholeukemia cells in

vitro (Wickstrom, E. L., et al., Proc. Natl. Acad. Sci. USA, 85:1028-1032, 1988). The sequentes shown in Table 6 were prepared and evaluated by the procedures of Example 41a and 41b.

Detailed Description Text (293):
41a. Comparison of the Effect of Modified (with TEG) and Non-Modified C-MYC Antisense DNA on the Progression of PHA Stimulated PBL Into the S-Phase of the Cell Cycle.

Detailed Description Text (294):
Human PBL's were stimulated with PHA for 48 hours in the presence or absence of the antisense oligonucleotide sequences of Table 6. The percent of the population of cells in each treatment group in the S-phase of the cell cycle as compared to the nontreated control was determined using standard flow cytometric techniques. The results are shown in Table 7.

Detailed Description Text (295):
The data show that the presence of TEG at both the 3' and 5' termini does not alter the inhibitory effect of the antisense DNA.

Detailed Description Text (296):
41b. comparison of the Effect of Modified (with TEG) and Non-Modified C-MYC Antisense DNA on C-MYC Protein Expression in MOLT-4 Human T-Cell Leukemia Cells.

Detailed Description Text (297):
Asynchronous exponentially growing Molt-4 cells were incubated for 8 hours in the presence or absence of 60 .mu.M c-myc directed antisense DNA. The cells were then incubated for 45 minutes in the presence of .sup.35 S-methionine and the content of c-myc protein guantitated using radioimmunoprecipitation with a c-myc antibody. The results are displayed in Table 8.

Detailed Description Text (298):
The TEG containing antisense DNA was slightly more potent than the unmodified antisense DNA.

Detailed Description Text (299):
41c. Comparison of the Effect of Modified (with TEG) and Unmodified C-MYC Antisense DNA to Inhibit the Growth of Human CCRF-CEM T-Cell Leukemia Cell Growth in Vitro.

Detailed Description Text (300):
Asynchronous exponentially growing CCRF-CEM cells were incubated for 48 hours in the presence or absence of antisense DNA and then cell numbers determined in each treatment group. The concentration of antisense DNA required to inhibit cell growth by 50% was then determined (IC.sub.50), Both of the modified and non-modified antisense DNAs of Table 5 displayed approximately equivalent (IC.sub.50) concentrations of 40 .mu.M.

Detailed Description Text (301):
These data demonstrate that the presence of TEG at the 3' and 5' termini of antisense DNA does not affect the ability of such antisense DNA to hybridize with and inhibit the function of target nucleic acids.

Other Reference Publication (1):
Gura, "antisense Has Growing Pains--Efforts to Develop Antisense Compounds for Cancer, AIDS, and Other Diseases Have Encountered Some Unexpectd Questions About How the Drugs Really Work," Science 270, 575-577 (1995).

Other Reference Publication (3):
Uhlmann et al., "Antisense Oligonucleotides: A New Therapeutic Principle," Chem. Reviews, 90(4), 543-584 (1990).

Other Reference Publication (4):
Cohen et al., "Antisense Oligonucleotides as Therapeutic Agents--Is the Bullet Really Magical?" Science, 261, 1004-1012 (1993).

Other Reference Publication (7):
Goodchild et al., "Inhibition of Replication and Expresion of HIV-1 Tissue Culture by Oligodeoxynucleotide Hybridization Competition," in Human Retroviruses, Cancer, and AIDS: Approaches to Prevention and Therapy, Alan R. Liss, Inc., New York, NY, 1988, pp. 423-438.

Other Reference Publication (10):
Vaseur et al., "Oligonucleosides: Synthesis of a Novel Methylhydroxylamine-Linked Nucleoside Dimer and Its Incorporation into Antisense Sequences," J. Am. Chem. Soc., 114, 4006-4007 (1992).

☐ 12. Document ID: US 5567810 A

L3: Entry 12 of 13 File: USPT Oct 22, 1996

DOCUMENT-IDENTIFIER: US 5567810 A
TITLE: Nuclease resistant compounds

Brief Summary Text (4):
Antisense compounds contain oligonucleotides that bind to or hybridize with a complementary nucleotide sequence in another nucleic acid, RNA or DNA, to inhibit the function or synthesis of said nucleic acid. Because of their ability to hybridize with both RNA and DNA, antisense compounds can interfere with gens expression at the level of transcription, RNA processing or translation.

Brief Summary Text (5):
Antisense compounds can be designed and synthesized to prevent the transcription of specific genes to RNA by hybridizing with genomic DNA and directly or indirectly inhibiting the action of RNA polymerass. An advantage of targeting DNA is that only small amounts of antisense compounds are needed to achieve a therapeutic effect. Alternatively, antisense compounds can be designed and synthesized to hybridize with with RNA to inhibit post-transcriptional modification (RNA processing) or protein synthesis (translation) mechanisms. Exemplary target RNAs are messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA) and the like. Examples of processing and translation mechanisms include splicing of pre-mRNA to remove introns, capping of the 5' terminus of mRNA, hybridization arrest and nuclease mediated mRNA hydrolysis.

Brief Summary Text (6):
At the present time, however, the development of practical scientific and therapeutic applications of antisense technologies is hampered by a number of technical problems. Klausner, A., Biotechnology, 8:303-304 (1990). Synthetic antisense molecules are susceptible to rapid degradation by nucleases that exist in target cells. The oligonucleotide sequences of antisense DNA or RNA, for example, are destroyed by exonucleases acting at either the 5' or 3' terminus of the nucleic acid. In addition, endonucleases can cleave the DNA or RNA at internal phosphodiester linkages between individual nucleotides. As a result of such cleavage, the effective half-life of administered antisense compounds is very short

necessitating the use of large, frequently administered dosages.

Brief Summary Text (7):
Another problem is the extremely high cost of producing antisense compounds using available semiautomatic nucleic acid synthesizers. It has recently been estimated that the cost of producing one gram of antisense DNA is about $100,000. Armstrong, L., Business Week, Mar. 5, 1990, page 89.

Brief Summary Text (8):
A further problem relates to the delivery of antisense agents to desired targets within the body and cell. Antisense agents targeted to genomic DNA must gain access to the nucleus (i.e. the agents must permeate the plasma and nuclear membrane). The need for increased membrane permeability (increased hydrophobicity) must be balanced, however, against the need for aqueous solubility (increased hydrophilicity) in body fluid compartments such as the plasma and cell cytosol.

Brief Summary Text (9):
A still further problem relates to the stability of antisense agents whether free within the body or hybridized to target nucleic acids. Oligonucleotides such as antisense DNA or RNA are susceptible to unstable steric reconfiguration around chiral phosphate centers.

Brief Summary Text (10):
Gene targeting via antisense agents is the inevitable next step in human therapeutics. Armstrong, supra at 88. The successful application of antisense technology to the treatment of disease however, requires finding solutions to the problems set forth above. The present invention provides compounds, compositions and methods for inhibiting nuclease degradation of antisense compounds.

Detailed Description Text (12):
In a preferred embodiment, the antisense compounds have polyethyleneglycols (PEGs) at both the 5' and 3' termini and have the formula:

Detailed Description Text (16):
The compounds of the present invention are useful as antisense agents. Antisense compounds contain aligonucleotide sequences that hybridize with a complementary nucleotide sequence in another target nucleic acid to inhibit the translational or transcriptional function of said target nucleic acid. The target nucleic acid may be either RNA or DNA.

Detailed Description Text (17):
Antisense DNA compounds of the present invention comprise oligonucleotides of from about 9 to about 200 bases having homopolymer or heteropolymer sequences of deoxynucleotides selected from the group consisting of deoxyadenylic acid (dA), deoxycytidylic acid (dC), deoxyguanylic acid (dG) or thymidylic acid (T).

Detailed Description Text (18):
Antisense RNA compounds of the present invention comprise oligonucleotides of from about 9 to about 200 bases having homopolymer or heteropolymer sequences of nucleotides selected from the group consisting of adenylic acid (A), cytidylic acid (C), guanylic acid (G) or uridylic acid (U).

Detailed Description Text (29):
Those skilled in the art will appreciate that other means of synthesizing oligonucleotides can be modified in an analogous manner to produce diol-terminated antisense oligonucleotides.

Detailed Description Text (30):
The compounds of the present invention are useful in treating mammals with hereditary disorders or diseases associated with altered genetic expression

mechanisms. At present, attempts are underway to develop antisense therapies for use use in treating viral infections such as HIV, cytomegalovirus, herpes simplex, hepatitis B, papilloma virus and picorna virus; cancers of the lung, cervix, colon, breast and ovary; inflammatory diseases; and diseases of the immune system such as acquired immunodeficiency syndrome (AIDS), hematological neoplasma, and hyperproliferative disorders. Armstrong, supra at 89; Klausner, Supra at 303, 304.

Detailed Description Text (44):
Compositions for rectal administrations are preferably suppositories which can be prepared by mixing the compounds of the present invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethyleneglycol or a suppository wax, which are solid at ordinary temperatures but liquid at body temperature and therefore, melt in the rectum or vaginal cavity and release the active component.

Detailed Description Text (77):
Ability of TEG-Antisense Oligomers to Inhibit Protein Expression and Growth in Human Human Tumor Cell Lines and PHA Stimulation of Peripheral Blood Lymphocytes.

Detailed Description Text (78):
It has been demonstrated by others (Heikkila, R. et al., Nature, 328:445-449, 1987) that unmodified antisense oligonucleotides directed towards the initiation codon region of the c-myc oncogene could inhibit the expression of c-myc protein in PHA stimulated peripheral blood lymphocytes (PBL) resulting in a block in the progression of cells into the S-phase of the cell cycle. C-myc directed antisense DNA was also shown to inhibit the growth of HL-60 human erytholeukemia cells in vitro (Wickstrom, E. L., et al., Proc. Natl. Acad. Sci. USA, 85:1028-1032, 1988). We directly compared the sequences of Table 4 for their ability to inhibit tumor cell growth, to downregulate c-myc expression, and to inhibit the progression of PHA stimulated PBL into the S-phase of the cell cycle.

Detailed Description Text (79):
a. Comparison of the Effect of Modified (with TEG) and Non-Modified C-MYC Antisense DNA on the Progression of PHA Stimulated PBL Into the S-Phase of the Cell Cycle.

Detailed Description Text (80):
Human PBL's were stimulated with PHA for 48 hours in the presence or absence of the antisense oligonucleotide sequences of Table 4. The percent of the population of cells in each treatment group in the S-phase of the cell cycle as compared to the nontreated control was determined using standard flow cytometric techniques. The results are shown in Table 5.

Detailed Description Text (81):
The data show that the presence of TEG at both the 3' and 5' termini does not alter the inhibitory effect of the antisense DNA.

Detailed Description Text (82):
b. Comparison of the Effect of Modified (with TEG) and Non-Modified C-MYC Antisense DNA on C-MYC Protein Expression in MOLT-4 Human T-Cell Leukemia Cells.

Detailed Description Text (83):
Asynchronus exponentially growing Molt-4 cells were incubated for 8 hours in the presence or absence of 60 .mu.M c-myc directed antisense DNA. The cells were then incubated for 45 minutes in the presence of .sup.35 S-methionine and the content of c-myc protein quantitated using radioimmunoprecipitation with a c-myc antibody.

Detailed Description Text (85):
The TEG containing antisense DNA was slightly more potent than the unmodified antisense DNA.

Detailed Description Text (86):
c. Comparison of the Effect of Modified (with TEG) and Unmodified C-MYC Antisense DNA to Inhibit the Growth of Human CCRF-CEM T-Cell Leukemia Cell Growth in Vitro.

Detailed Description Text (87):
Asynchronus exponentially growing CCRF-CEM cells were incubated for 48 hours in the presence or absence of antisense DNA and then cell numbers determined in each treatment group. The concentration of antisense DNA required to inhibit cell growth by 50% was then determined (IC.sub.50). Both of the modified and non-modified antisense DNAs of Table 3 displayed approximately equivalent (IC.sub.50) concentrations of 40 .mu.M.

Detailed Description Text (88):
These data demonstrate that the presence of TEG at the 3' and 5' termini of antisense DNA does not affect the ability of such antisense DNA to hybridize with and inhibit the function of target nucleic acids.

Detailed Description Paragraph Table (4):
TABLE 4 _____ UNMODIFIED C-MYC ANTISENSE SEQUENCE 5' ACC GTT GAG GGG CAT 3' MODIFIED C-MYC ANTISENSE SEQUENCE 5' XX AAC GTT GAG GGG CAT XX A 3' _____ (X = TEG)

Other Reference Publication (1):
Gura Science 270 575-577 (1995) Antisense has growing pains.

☐ 13. Document ID: US 5245022 A

L3: Entry 13 of 13 File: USPT Sep 14, 1993

DOCUMENT-IDENTIFIER: US 5245022 A
TITLE: Exonuclease resistant terminally substituted oligonucleotides

Brief Summary Text (4):
Antisense compounds contain oligonucleotides that bind to or hybridize with a complementary nucleotide sequence in another nucleic acid, RNA or DNA, to inhibit the function or synthesis of said nucleic acid. Because of their ability to hybridize with both RNA and DNA, antisense compounds can interfere with gene expression at the level of transcription, RNA processing or translation.

Brief Summary Text (5):
Antisense compounds can be designed and synthesized to prevent the transcription of specific genes to RNA by hybridizing with genomic DNA and directly or indirectly inhibiting the action of RNA polymerase. An advantage of targeting DNA is that only small amounts of antisense compounds are needed to achieve a therapeutic effect. Alternatively, antisense compounds can be designed and synthesized to hybridize with with RNA to inhibit post-transcriptional modification (RNA processing) or protein synthesis (translation) mechanisms. Exemplary target RNAs are messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA) and the like. Examples of processing and translation mechanisms include splicing of pre-mRNA to remove introns, capping of the 5' terminus of mRNA, hybridization arrest and nuclease mediated mRNA hydrolysis.

Brief Summary Text (6):

At the present time, however, the development of practical scientific and therapeutic applications of antisense technologies is hampered by a number of technical problems. Klausner, A., Biotechnology, 8:303-304 (1990). Synthetic antisense molecules are susceptible to rapid degradation by nucleases that exist in target cells. The oligonucleotide sequences of antisense DNA or RNA, for example, are destroyed by exonucleases acting at either the 5' or 3' terminus of the nucleic acid. In addition, endonucleases can cleave the DNA or RNA at internal phosphodiester linkages between individual nucleotides. As a result of such cleavage, the effective half-life of administered antisense compounds is very short necessitating the use of large, frequently administered dosages.

Brief Summary Text (7):
Another problem is the extremely high cost of producing antisense compounds using available semiautomatic nucleic acid synthesizers. It has recently been estimated that the cost of producing one gram of antisense DNA is about $100,000. Armstrong, L., Business Week, Mar. 5, 1990, page 89.

Brief Summary Text (8):
A further problem relates to the delivery of antisense agents to desired targets within the body and cell. Antisense agents targeted to genomic DNA must gain access to the nucleus (i.e. the agents must permeate the plasma and nuclear membrane). The need for increased membrane permeability (increased hydrophobicity) must be balanced, however, against the need for aqueous solubility (increased hydrophilicity) in body fluid compartments such as the plasma and cell cytosol.

Brief Summary Text (9):
A still further problem relates to the stability of antisense agents whether free within the body or hybridized to target nucleic acids. Oligonucleotides such as antisense DNA or RNA are susceptible to unstable steric reconfiguration around chiral phosphate centers.

Brief Summary Text (10):
Gene targeting via antisense agents is the inevitable next step in human therapeutics. Armstrong, supra at 88. The successful application of antisense technology to the treatment of disease however, requires finding solutions to the problems set forth above. The present invention provides compounds, compositions and methods for inhibiting nuclease degradation of antisense compounds.

Detailed Description Text (15):
In a preferred embodiment, the antisense compounds have polyethyleneglycols (PEGs) at both the 5' and 3' termini and have the formula:

Detailed Description Text (23):
The compounds of the present invention are useful as antisense agents. Antisense compounds contain oligonucleotide sequences that hybridize with a complementary nucleotide sequence in another target nucleic acid to inhibit the translational or transcriptional function of said target nucleic acid. The target nucleic acid may be either RNA or DNA.

Detailed Description Text (24):
Antisense DNA compounds of the present invention comprise oligonucleotides of from about 9 to about 200 bases having homopolymer or heteropolymer sequences of deoxynucleotides selected from the group consisting of deoxyadenylic acid (dA), deoxycytidylic acid (dC), deoxyguanylic acid (dG) or thymidylic acid (T).

Detailed Description Text (25):
Antisense RNA compounds of the present invention comprise oligonucleotides of from about 9 to about 200 bases having homopolymer or heteropolymer sequences of nucleotides selected from the group consisting of adenylic acid (A), cytidylic acid (C), guanylic acid (G) or uridylic acid (U).

Detailed Description Text (36):
Those skilled in the art will appreciate that other means of synthesizing oligonucleotides can be modified in an analogous manner to produce diol-terminated antisense oligonucleotides.

Detailed Description Text (37):
The compounds of the present invention are useful in treating mammals with hereditary disorders or diseases associated with altered genetic expression mechanisms. At present, attempts are underway to develop antisense therapies for use in treating viral infections such as HIV, cytomegalovirus, herpes simplex, hepatitis B, papilloma virus and picorna virus; cancers of the lung, cervix, colon, breast and ovary; inflammatory diseases; and diseases of the immune system such as acquired immunodeficiency syndrome (AIDS), hematological neoplasma, and hyperproliferative disorders. Armstrong, supra at 89; Klausner, supra at 303, 304.

Detailed Description Text (51):
Compositions for rectal administrations are preferably suppositories which can be prepared by mixing the compounds of the present invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethyleneglycol or a suppository wax, which are solid at ordinary temperatures but liquid at body temperature and therefore, melt in the rectum or vaginal cavity and release the active component.

Detailed Description Text (80):
Ability of TEG-Antisense Oligomers to Protein Expression and Growth in Human Tumor Cell Lines and PHA Stimulation of Peripheral Blood Lymphocytes

Detailed Description Text (81):
It has been demonstrated by others (Heikkila, R. et al., Nature, 328:445-449, 1987) that unmodified antisense oligonucleotides directed towards the initiation codon region of the c-myc oncogene could inhibit the expression of c-myc protein in PHA stimulated peripheral blood lymphocytes (PBL) resulting in a block in the progression of cells into the S-phase of the cell cycle. C-myc directed antisense DNA was also shown to inhibit the growth of HL-60 human erytholeukemia cells in vitro (Wickstrom, E. L., et al., Proc. Natl. Acad. Sci. USA, 85:1028-1032, 1988). We directly compared the sequences of Table 4 for their ability to inhibit tumor cell growth, to downregulate cmyc expression, and to inhibit the progression of PHA stimulated PBL into the S-phase of the cell cycle.

Detailed Description Text (82):
a. Comparison of the Effect of Modified (with TEG) and Non-Modified C-MYC Antisense DNA on the Progression of PHA Stimulated PBL Into the S-Phase of the Cell Cycle

Detailed Description Text (83):
Human PBL's were stimulated with PHA for 48 hours in the presence or absence of the antisense oligonucleotide sequences of Table 4. The percent of the population of cells in each treatment group in the S-phase of the cell cycle as compared to the nontreated control was determined using standard flow cytometric techniques. The results are shown in Table 5.

Detailed Description Text (84):
The data show that the presence of TEG at both the 3' and 5' termini does not alter the inhibitory effect of the antisense DNA.

Detailed Description Text (85):
b. Comparison of the Effect of Modified (with TEG) and Non-Modified C-MYC Antisense DNA on C-MYC Protein Expression in MOLT-4 Human T-Cell Leukemia Cells

Detailed Description Text (86):

Asynchronus exponentially growing Molt-4 cells were incubated for 8 hours in the presence or absence of 60 .mu.M c-myc directed antisense DNA. The cells were then incubated for 45 minutes in the presence of .sup.35 S-methionine and the content of c-myc protein quantitated using radioimmunoprecipitation with a c-myc antibody.

Detailed Description Text (88):
The TEG containing antisense DNA was slightly more potent than the unmodified antisense DNA.

Detailed Description Text (89):
c. Comparison of the Effect of Modified (with TEG) and Unmodified C-MYC Antisense DNA to Inhibit the Growth of Human CCRF-CEM T-Cell Leukemia Cell Growth in Vitro

Detailed Description Text (90):
Asynchronus exponentially growing CCRF-CEM cells were incubated for 48 hours in the presence or absence of antisense DNA and then cell numbers determined in each treatment group. The concentration of antisense DNA required to inhibit cell growth by 50% was then determined (IC.sub.50) Both of the modified and non-modified antisense DNAs of Table 3 displayed approximately equivalent (IC.sub.50) concentrations of 40 .mu.M.

Detailed Description Text (91):
These data demonstrate that the presence of TEG at the 3' and 5' termini of antisense DNA does not affect the ability of such antisense DNA to hybridize with and inhibit the function of target nucleic acids.

Detailed Description Paragraph Table (4):
TABLE 4 UNMODIFIED C-MYC ANTISENSE SEQUENCE 5' ACC GTT GAG GGG CAT 3' MODIFIED C-MYC ANTISENSE SEQUENCE 5' XX AAC GTT GAG GGG CAT XX A 3' (X = TEG)

Terms	Documents
L1 and (supposit$ same (peg$ or peg or polyethyleneglycol or poly-glycol or poly-ethylene-glycol))	13

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L11: Entry 1 of 7 File: USPT Nov 11, 2003

US-PAT-NO: 6645528
DOCUMENT-IDENTIFIER: US 6645528 B1
**** See image for Certificate of Correction ****

TITLE: Porous drug matrices and methods of manufacture thereof

DATE-ISSUED: November 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Bernstein; Howard	Cambridge	MA		
Chickering, III; Donald E.	Framingham	MA		
Khattak; Sarwat	Cambridge	MA		
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ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Acusphere, Inc.	Cambridge	MA			02

APPL-NO: 09/ 694407 [PALM]
DATE FILED: October 23, 2000

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This is a divisional of U.S. application Ser. No. 09/433,486, file Nov. 4, 1999, now U.S. Pat No. 6,395,300 which claims priority of 60/136323 filed May 27, 1999 and 60/158659 Oct. 8, 1999.

INT-CL: [07] A61 K 9/14

US-CL-ISSUED: 424/489; 514/951
US-CL-CURRENT: 424/489; 514/951

FIELD-OF-SEARCH: 424/484, 424/489, 428/402, 514/951-52

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	5382437	January 1995	Ecanow	

First Hit Fwd Refs

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L11: Entry 1 of 7 File: USPT Nov 11, 2003

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Chickering, III; Donald E.	Framingham	MA		
Khattak; Sarwat	Cambridge	MA		
Randall; Greg	Stoneham	MA		

US-CL-CURRENT: 424/489; 514/951

CLAIMS:

We claim:

1. A pharmaceutical composition comprising a porous matrix which comprises a wetting agent and microparticles of a drug, wherein the microparticles a mean diameter between about 0.1 and 5 .mu.m and a total surface area greater than about 0.5 m.sup.2 /mL, and wherein the porous matrix has a TAP density less than or equal to 1.0 g/mL and/or has a total surface area of greater than or equal to 0.2 m.sup.2 /g and in the form of a powder.

2. The composition of claim 1 wherein the drug is a low aqueous solubility drug.

3. The composition of claim 2 wherein the porous matrix is in an aqueous medium.

4. The composition of claim 3 wherein the drug is selected from the group consisting of albuterol, adapalene, doxazosin mesylate, mometasone furoate, ursodiol, amphotericin, enalapril maleate, felodipine, nefazodone hydrochloride, valrubicin, albendazole, conjugated estrogens, medroxyprogesterone acetate, nicardipine hydrochloride, zolpidem tartrate, amlodipine besylate, ethinyl estradiol, omeprazole, rubitecan, amlodipine besylate/benazepril hydrochloride, etodolac, paroxetine hydrochloride, paclitaxel, atovaquone, felodipine, podofilox, paricalcitol, betamethasone dipropionate, fentanyl, pramipexole dihydrochloride, Vitamin D.sub.3, finasteride, quetiapine fumarate, alprostadil candesartan, cilexetil, fluconazole, ritonavir, busulfan, carbamazepine, flumazenil, risperidone, carbemazepine, carbidopa/levodopa, ganciclovir, saquinavir, amprenavir, carboplatin, glyburide, sertraline hydrochloride, rofecoxib carvedilol, halobetasolproprionate, sildenafil citrate, celecoxib, chlorthalidone, imiquimod, simvastatin,

citalopram, ciprofloxacin, irinotecan hydrochloride, sparfloxacin, efavirenz, cisapride monohydrate, lansoprazole, tamsulosin hydrochloride, mofafinil, azithromycin, clarithromycin, letrozole, terbinafine hydrochloride, rosiglitazone maleate, diclofenac sodium, lomefloxacin hydrochloride, tirofiban hydrochloride, telmisartan, diazapam, loratadine, toremifene citrate, thalidomide, dinoprostone, mefloquine hydrochloride, trandolapril, docetaxel, mitoxantrone hydrochloride, tretinoin, etodolac, triamcinolone acetate, estradiol, ursodiol, nelfinavir mesylate, indinavir, beclomethasone dipropionate, oxaprozin, flutamide, famotidine, nifedipine, prednisone, cefuroxime, lorazepam, digoxin, lovastatin, griseofulvin, naproxen, ibuprofen, isotretinoin, tamoxifen citrate, nimodipine, amiodarone, and alprazolam.

5. The composition of claim 1 wherein the drug is water soluble.

6. The composition of claim 5 wherein the drug is selected from the group consisting of ceftriaxone, ketoconazole, ceftazidime, oxaprozin, valacyclovir urofollitropin, famciclovir, flutamide, enalapril, mefformin, itraconazole, buspirone, gabapentin, fosinopril, tramadol, acarbose, lorazepan, follitropin, glipizide, omeprazole, fluoxetine, lisinopril, levofloxacin, zafirlukast, interferon, growth hormone, interleukin, erythropoietin, granulocyte stimulating factor, nizatidine, bupropion, perindopril, erbumine, adenosine, alendronate, alprostadil, benazepril, betaxolol, bleomycin sulfate, dexfenfluramine, diltiazem, fentanyl, flecainid, gemcitabine, glatiramer acetate, granisetron, lamivudine, mangafodipir trisodium, mesalamine, metoprolol fumarate, metronidazole, miglitol, moexipril, monteleukast, octreotide acetate, olopatadine, paricalcitol, somatropin, sumatriptan succinate, tacrine, verapamil, nabumetone, trovafloxacin, dolasetron, zidovudine, finasteride, tobramycin, isradipine, tolcapone, enoxaparin, fluconazole, lansoprazole, terbinafine, pamidronate, didanosine, diclofenac, cisapride, venlafaxine, troglitazone, fluvastatin, losartan, imiglucerase, donepezil, olanzapine, valsartan, fexofenadine, calcitonin, and ipratropium.

7. The composition of claim 1 wherein the matrix further comprise an excipient selected from the group consisting of hydrophilic polymers, sugars, tonicity agents, pegylated excipients, and combinations thereof.

8. The composition of claim 1 wherein the mean diameter of the microparticles is between about 1 and 5 .mu.m.

9. The composition of claim 1 wherein the microparticles are suspended in an aqueous solution suitable for parenteral administration.

10. The composition of claim 1 wherein the matrix is processed into tablets or capsules suitable for oral administration.

11. The composition of claim 1 wherein the matrix is formed into suppositories suitable for vaginal or rectal administration.

12. The composition of claim 1 wherein the matrix is in a dry powder form suitable for pulmonary administration.

13. The composition of claim 1 wherein the dry powder form of the porous matrix has a TAP density less than or equal to 1.0 g/mL.

14. The composition of claim 1 wherein the dry powder form of the porous matrix has a total

surface of greater than or equal to 0.2 m.sup.2 /g.

15. The composition of claim 1 wherein the mean diameter of the microparticles is between about 0.5 end 5 .mu.m.

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ART-UNIT: 1617

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ATTY-AGENT-FIRM: Holland & Knight LLP

ABSTRACT:

Drugs, especially low aqueous solubility drugs, are provided in a porous matrix form, preferably microparticles, which enhances dissolution of the drug in aqueous media. The drug matrices preferably are made using a process that includes (i) dissolving a drug, preferably a drug having low aqueous solubility, in a volatile solvent to form a drug solution, (ii) combining at least one pore forming agent with with the drug solution to form an emulsion, suspension, or second solution, and (iii) removing the volatile solvent and pore forming agent from the emulsion, suspension, or second solution to yield the porous matrix of drug. The pore forming agent can be either a volatile liquid that is immiscible with the drug solvent or a volatile solid compound, preferably a volatile salt. In a preferred embodiment, spray drying is used to remove the solvents and the pore forming agent. The resulting porous matrix has a faster rate of dissolution following administration to to a patient, as compared to non-porous matrix forms of the drug. In a preferred embodiment, microparticles of the porous drug matrix are reconstituted with an aqueous medium and administered parenterally, or processed using standard techniques techniques into tablets or capsules for oral administration.

15 Claims, 8 Drawing figures

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L8: Entry 1 of 4 File: USPT May 25, 2004

US-PAT-NO: 6740333
DOCUMENT-IDENTIFIER: US 6740333 B2

TITLE: Suppository and composition comprising at least one polyethylene glycol

DATE-ISSUED: May 25, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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ASSIGNEE-INFORMATION:

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APPL-NO: 09/ 899567 [PALM]
DATE FILED: July 6, 2001

PARENT-CASE:
The present application claims the benefit of U.S. Provisional Application No. 60/256,923, filed Dec. 21, 2000, hereby incorporated by reference in its entirety. It also claims the benefit of Danish applications PA 2000 01067 (Jul. 7, 2000), PA 2000 01923 (Dec. 21, 2000), and PA 2001 01050 (Jul 3, 2001), also incorporated by reference in their entirety. Finally, all patents, patent applications and publications cited herein are incorporated by reference in their entirety.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	200001067	July 7, 2000
DK	200001923	December 21, 2000
DK	200101050	July 3, 2001

INT-CL: [07] A61 F 9/02

US-CL-ISSUED: 424/436; 424/DIG.5, 514/965, 514/966
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FIELD-OF-SEARCH: 424/436, 424/DIG.15, 514/965, 514/966

PRIOR-ART-DISCLOSED:

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ART-UNIT: 1615

PRIMARY-EXAMINER: Azpuru; Carlos A.

ATTY-AGENT-FIRM: Cooper; Iver P.

ABSTRACT:

There is provided a suppository comprising at least one biocompatible polymer, wherein the biocompatible polymer is essentially non-biodegradable, and wherein the suppository essentially does not swell when contacted with an aqueous fluid. The suppository may further comprise a plurality of open cells at least partly separated separated from one another by an interpenetrating matrix comprising at least one biocompatible polymer in branched or crosslinked form. The plurality of interlinked, interlinked, open cells are capable of containing an aqueous fluid, and the permeability of the suppository ensures that entry of body fluids into the open cells under practical circumstances occurs essentially without dehydration of mucousal membrane tissue contacting the suppository. The suppository furthermore preferably comprises a controlled release formulation.

155 Claims, 20 Drawing figures

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L8: Entry 1 of 4 File: USPT May 25, 2004

US-PAT-NO: 6740333
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TITLE: Suppository and composition comprising at least one polyethylene glycol

DATE-ISSUED: May 25, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Eliasen; Per Robert Topp	Koersoer			DK

US-CL-CURRENT: 424/436; 424/DIG.5, 514/965, 514/966

CLAIMS:

What is claimed is:

1. Suppository for administration of at least one bioactive substance, said suppository comprising i) a polymer composition comprising at least one biocompatible polymer, wherein the biocompatible polymer is essentially non-biodegradable; and ii) a controlled release formulation for controlled release of said at least one bioactive substance, said formulation comprising at least one biodegradable polymer; and wherein the suppository essentially does not swell when contacted with an aqueous fluid.

2. The suppository according to claim 1, wherein the polymer compositions has a density of from about 100 gram per litre to about 250 gram per litre.

3. The suppository according to claim 1, wherein the compression modulus of the polymer composition at ambient temperature is from about 10 kPa to about 30 kPa.

4. The suppository according to claim 1, wherein the polymer composition further comprises a mixture of cells selected from the group consisting of open cells and closed cells.

5. The suppository according to claim 4, wherein the majority of the cells are open cells.

6. The suppository according to claim 5, wherein more than about 90% of the cells are open cells.

7. The suppository according to claim 6, wherein essentially all the cells are open cells.

8. The suppository according to claim 1, wherein the polymer composition further comprises a plurality of open cells at least partly separated from one another by an interpenetrating matrix comprising at least one biocompatible polymer in branched or crosslinked form.

9. The suppository according to claim 8, wherein at least part of the polymer composition comprises a plurality of interlinked, open cells capable of containing an aqueous fluid.

10. The suppository according to claim 9, wherein the contacting of the suppository with an aqueous fluid under practical circumstances results in essentially no fluid entering the open cells.

11. The suppository according to claim 10, wherein the permeability of the polymer composition results in entry of fluid into the open cells under practical circumstances essentially without dehydration of mucousal membrane tissue contacting the suppository.

12. The suppository according to claim 1, wherein the glass-rubber transition temperature of the polymer of the polymer composition is above about 15.degree. C. and preferably below 40.degree. C., such as a glass rubber transition temperature in the range of about 20.degree. C. to about 40.degree. C., for example about 35.degree. C., such as about 36.degree. C., for example in the range of about 25.degree. C. to about 35.degree. C., such as in the range of about 30.degree. C. to about 35.degree. C.

13. The suppository according to claim 1, wherein the biocompatible polymer is selected from the group consisting of polyurethanes, polyethylenes, and polypropylenes.

14. The suppository according to claim 1, wherein the biocompatible polymer is a polyurethane.

15. The suppository according to claim 14, wherein the polyurethane comprises at least one polyisocyanat that is at least partly polymerised with at least one polyol.

16. The suppository according to claim 15, wherein essentially all of the at least one polyisocyanat is polymerised with essentially all of the at least one polyol.

17. The suppository according to claim 15, wherein the at least one polyisocyanat is selected from the group consisting of aromatic polyisocyanates, aliphatic polyisocyanates, and heterocyclic polyisocyanates.

18. The suppository according to claim 15, wherein the at least one polyol is selected from the group consisting of aromatic polyols, aliphatic polyols, and heterocyclic polyols.

19. The suppository according to claim 15, wherein the at least one polyol is aliphatic and the at least one polyisocyanat is aliphatic.

20. The suppository according to claim 15, wherein the at least one polyol is aliphatic, such as a polymer comprising or essentially consisting of 2,2-dihydroxy-dipropylether, and the at least one polyisocyanat is aromatic, such as a polymer comprising or essentially consisting of diphenylmethandiisocyanat.

21. The suppository according to claim 15, wherein the at least one polyol is aromatic and the at least one polyisocyanat is aliphatic.

22. The suppository according to claim 15, wherein the at least one polyol is aromatic and the at least one polyisocyanat is aromatic.

23. The suppository according to claim 15, comprising two polyols.

24. The suppository according to claim 15, wherein the polyol is selected from the group consisting of diols and triols.

25. The suppository according to claim 23, wherein the average functionality of the at least one polyol is between 0.2 and 5, preferably between 0.5 and 2.

26. The suppository according to claim 15, wherein the ratio of i) NCO groups contained in the at least one polyisocyanate to ii) OH groups contained in the at least one polyol, respectively, is within the range of from about 0.5 to about 2.5, and preferably within the range of about 0.7 to about 1.3.

27. The suppository according to claim 1, wherein the controlled release formulation has a softening point between 15.degree. C. and 45.degree. C.

28. The suppository according to claim 1, wherein the controlled release formulation has a softening point between 25.degree. C. and 40.degree. C.

29. The suppository according to claim 1, wherein the controlled release formulation has a softening point between 30.degree. C. and 35.degree. C.

30. The suppository according to claim 1, wherein the controlled release formulation has a softening point such as the suppository is essentially rigid at room temperature, and the suppository is essentially soft at body temperature.

31. The suppository according to claim 1, wherein the controlled release formulation comprises two different biodegradable polymers.

32. The suppository according to claim 1, wherein the controlled release formulation comprises more than two different biodegradable polymers.

33. The suppository according to claim 1, wherein at least one polymer of the controlled release formulation is selected from the group consisting of polyethyleneglycols (PEG).

34. The suppository according to claim 33, wherein the PEG has an average molecular weight of between 100 and 1500.

35. The suppository according to claim 33, wherein the PEG has an average molecular weight of between 400 and 1000.

36. The suppository according to claim 33, wherein the PEG has an average molecular weight of around 400.

37. The suppository according to claim 33, wherein the PEG has an average molecular weight of around 1000.

38. The suppository according to claim 33, wherein the PEG has an average molecular weight of

more than 1000.

39. The suppository according to claim 33, wherein the PEG has an average molecular weight between 1000 and 35,000.

40. The suppository according to claim 33, wherein the PEG has an average molecular weight between 1500 and 10,000.

41. The suppository according to claim 33, wherein the PEG has an average molecular weight around 2000.

42. The suppository according to claim 33, wherein the PEG has an average molecular weight around 4000.

43. The suppository according to claim 33, wherein the PEG has an average molecular weight 6000.

44. The suppository according to claim 31, wherein the controlled release formulation comprises (1) a polymer which is selected from the group consisting of PEG with an average molecular weight of between 200 and 1500 and (2) a polymer which is selected from the group consisting of PEG with an average molecular weight of between 1000 and 35,000.

45. The suppository according to claim 44, wherein the polymer (1) is PEG with an average molecular weight around 1000 and the polymer (2) is PEG with an average molecular weight around 4000.

46. The suppository according to claim 1, wherein the controlled release formulation furthermore comprises a surface active agent.

47. The suppository according to claim 1, wherein the surface active agent is a PEG monoester.

48. The suppository according to claim 1, wherein the surface active agent is a PEG monostearate.

49. The suppository according to claim 1, wherein the suppository comprises a central core comprising a first composition, and a surrounding part surrounding the central core and comprising a second composition.

50. The suppository according to claim 49, wherein the central core comprises the controlled release formulation and the surrounding part comprises the polymer composition.

51. The suppository according to claim 49, wherein the density of the central core, measured as mass per unit volume, is higher than the density of the surrounding part, measured as mass per unit volume.

52. The suppository according to claim 49, that further comprises a peripheral part comprising a third composition that is not identical to any of said first and second compositions, and wherein at least part of the third composition is in contact with the surrounding part comprising the second composition.

53. The suppository according to claim 52, wherein said peripheral part is a layer controlling the water permeability of the suppository by defining a barrier between the suppository and fluid present in an external environment that is in contact with the suppository under practical circumstances.

54. The suppository according to claim 1 and further comprising a coating composition, wherein the coating composition covers at least part of the surface area of said suppository, and wherein the coating composition is not identical to the polymer.

55. The suppository according to claim 1, wherein the bioactive substance is a medicament.

56. The suppository according to claim 55, wherein the medicament is selected from the group consisting of analgetics.

57. The suppository according to claim 56, wherein the analgetic is lidocain.

58. The suppository according to claim 1, wherein the controlled release formulation comprises the bioreactive substance comprised in capsules comprising at least one encapsulation agent.

59. The suppository according to claim 56, wherein the capsules comprise an encapsulation agent selected from the group of encapsulation agents consisting of PEG, proteins, lipids, and casein.

60. The suppository according to claim 56, wherein the capsules are adapted to disintegrate and release the medicament when contracting body tissue.

61. The suppository according to claim 56, wherein the capsules comprising the medicament are adapted to disintegrate and release the medicament when contacting a fluid, including moisture secreted by body tissue.

62. The suppository according to claim 56, wherein the capsules are adapted to dissolve and release the medicament when contacting body tissue.

63. The suppository according to claim 56, wherein the capsules comprising the medicament are adapted to dissolve and release the medicament when contacting a fluid.

64. The suppository according to claim 1, that further comprises an additive selected from the group of preservatives, adjuvants, stabilizers, lubricants, and disintegraters or any combinations thereof.

65. The suppository according to claim 1, wherein the suppository has an essentially oblong shape.

66. The suppository according to claim 1, wherein the suppository has an essentially cylindrical shape.

67. The suppository according to claim 1, wherein the suppository has a central portion, a first end portion and a second end portion, wherein at least one of said end portions is tapering, and preferably pointed.

68. The suppository according to claim 1, wherein the suppository has a central portion, a tapering, distal end portion, and a proximal end portion attached to a flange portion extending outwardly in the axial direction from said proximal end portion.

69. The suppository according to claim 67, wherein said flange portion has a recessed part in the axial direction.

70. The suppository according to claim 69, wherein the suppository has a shape that allows contact between parts of or essentially all of the outer surface of the suppository and parts of or essentially all of the mucosal membrane of the rectum below the caudal semilunary fold, when the suppository is situated in the rectum.

71. Method for preparation of a suppository according to claim 1, said method comprising the steps of i) providing at least one monomeric reagent suitable for polymerization, ii) polymerising said at least one monomeric reagent and obtaining a biocompatible polymer which is essentially non-biodegradable, iii) providing a controlled release formulation for controlled release of at least one bioactive substance, iv) mixing the controlled release formulation and the at least one bioactive substance, and v) shaping the essentially non-biodegradable, biocompatible polymer and the mixture of the controlled release formulation and the at least one bioactive substance into a shape desirable for a suppository.

72. Method according to claim 71, wherein the suppository is shaped by injection moulding into a predetermined shape.

73. Method of claim 71, wherein the monomeric reagents used for obtaining the essentially non-biodegradable, biocompatible polymer comprise at least one polyisocyanate and at least one polyo.

74. Suppository obtainable by the method of claim 71.

75. Method for alleviating pain in an individual in need thereof, said method comprising the steps of contacting body tissue of a body cavity of the individual with the suppository of claim 1, wherein the suppository comprises the bioactive substance in an amount effective to achieve said pain alleviation in said individual.

76. A method for diagnosing a clinical indication in an individual comprising the steps of bringing a suppository according to claim 1 into contact with body tissue of a body cavity an animal, wherein the suppository comprises a diagnostically active substance in an amount effective to achieve said diagnosis.

77. A cosmetic method comprising the steps of bringing a suppository according to claim 1 into contact with body tissue of a body cavity of an animal, wherein the suppository comprises a cosmetically active substance in an amount sufficient to achieve a desirable cosmetic effect.

78. The suppository of claim 33, where the controlled release formulation comprises (a) a biodegradable polyethylene glycol with a molecular weight of between 100 and 1500, and (b) a biodegradable polyethylene glycol with a molecular weight of between 1500 and 10,000.

79. The suppository of claim 33, where the controlled release formulation comprises (a) a biodegradable polyethylene glycol with a molecular weight of between 400 and 1000, and (b) a

biodegradable polyethylene glycol with a molecular weight of more than 1000.

80. The suppository of claim 79 where PEG (b) has a molecular weight of not more than 35,000.

81. The suppository of claim 33 where at least two of the biodegradable polymers of said controlled release formulation differ in melting point from each other.

82. The suppository of claim 81 where the ratio of the lowest melting point biodegradable polymer to the highest melting point polymer of said controlled release formulation is about 3:1.

83. Method of claim 75, wherein the bioactive substance is selected from the group consisting of analgesics, anaesthetics and antipyretics.

84. Method of claim 75, wherein the bioactive substance is selected from opioid analgesics, non-opioid analgesics, and lidocaine, and antiepileptics used to alleviate pain.

85. Method of claim 75, wherein the bioactive substance is selected from lidocaine, codeine, morphine, acetaminophen, aspirin, and ibuprofen.

86. Method of claim 75, wherein the body tissue is a rnucosal surface.

87. Method of claim 86, wherein the mucosal surface is a mucosal surface of the rectum.

88. Method of claim 87, wherein the mucosal surface of the rectum is the surface located below the caudal semilunary fold.

89. Method of claim 75, wherein the individual is a human being.

90. In a method for surgical treatment of ruptured colon in an individual, the improvement comprising alleviating pain by performing the steps of contacting body tissue of a body cavity of the individual with the suppository of claim 1, wherein the suppository comprises an analgesic bioactive substance or an anaesthetic bioactive substance in an amount effective to achieve said pain alleviation in said individual.

91. Method of claim 90, wherein the body tissue is a mucosal surface.

92. Method of claim 91, wherein the mucosal surface is a mucosal surface of the rectum.

93. Method of claim 92, wherein the mucosal surface of the rectum is the surface located below the caudal semilunary fold.

94. Method of claim 90, wherein the individual is a human being.

95. In a method for rectal surgery performed on an individual, the improvement comprising alleviating pain by performing the steps of contacting body tissue of a body cavity of the individual with the suppository of claim 1, wherein the suppository comprises an analgesic bioactive substance or an anaesthetic bioactive substance in an amount effective to achieve said pain alleviation in said individual.

96. Method of claim 95, wherein the body tissue is a mucosal surface.

97. Method of claim 96, wherein the mucosal surface is a mucosal surface of the rectum.

98. Method of claim 97, wherein the mucosal surface of the rectum is the surface located below the caudal semilunary fold.

99. Method of claim 95, wherein the individual is a human being.

100. In a method for surgical treatment of haemorrhoids in an individual, the improvement comprising alleviating pain by performing the steps of contacting body tissue of a body cavity of the individual with the suppository of claim 1, wherein the suppository comprises an analgetic bioactive substance or an anaesthetic bioactive substance in an amount effective to achieve said pain alleviation in said individual.

101. Method of claim 100, wherein the body tissue is a mucosal surface.

102. Method of claim 101, wherein the mucosal surface is a mucosal surface of the rectum.

103. Method of claim 102, wherein the mucosal surface of the rectum is the surface located below the caudal semilunary fold.

104. Method of claim 100, wherein the individual is a human being.

105. In a method for surgical treatment of ruptured vagina of a female mammal, the improvement comprising alleviating pain by performing the steps of contacting a mucosal surface of the vagina of the female mammal with the suppository of claim 1, wherein the suppository comprises an analgetic bioactive substance or an anaesthetic bioactive substance in an amount effective to achieve said pain alleviation in said female mammal.

106. Method of claim 105, wherein the mammal is a human being.

107. A method for treating an inflammatory condition in an individual, said method comprising the steps of contacting body tissue of a body cavity of the individual with the suppository of claim 1, wherein the suppository comprises an anti-inflammatory bioactive substance in an amount effective in treating said inflammatory condition.

108. Method of claim 107, wherein the body tissue is a mucosal surface.

109. Method of claim 108, wherein the mucosal surface is a mucosal surface of the rectum.

110. Method of claim 109, wherein the mucosal surface of the rectum is the surface located below the caudal semilunary fold.

111. Method of claim 107, wherein the individual is a human being.

112. Method of claim 107, wherein the anti-inflammatory bioactive substance is selected from the group consisting of naproxyn, diclofenac, indomethacin, ibuprofen, acetaminophen, aspirin, and

sulindac.

113. Method of claim 107, wherein the anti-inflammatory bioactive substance is selected from the group consisting of hydrocortisone, triamcinolone, prednisone, cortisone acetate, prednisolone, methyl prednisolone and dexamethasone.

114. Method for treating an infection in an individual, said method comprising the steps of contacting body tissue of a body cavity of the individual with the suppository of claim 1, wherein the suppository comprises an anti-infective bioactive substance in an amount effective in treating said infection.

115. Method of claim 114, wherein the body tissue is a mucosal surface.

116. Method of claim 115, wherein the mucosal surface is a mucosal surface of the rectum.

117. Method of claim 116, wherein the mucosal surface of the rectum is the surface located below the caudal semilunary fold.

118. Method of claim 114, wherein the individual is a human being.

119. Method of claim 114, wherein the anti-infective bioactive substance is selected from the group consisting of anti-biotics, anti-fungals, anti-virals, and anti-septics and anti-protozoans.

120. Method of claim 114, wherein the anti-biotic is selected from the group consisting of penicillins, cephalosporins, tetracyclines, ampicillin, aureothicin, bacitracin, chioramphenicol, cycloserine, erythromycin, gentamicin, gramacidin, kanamycin, neomycin, streptomycin, tobramycin, vancomycin, and metronidazole.

121. Method of claim 114, wherein the anti-biotic is a beta-lactam antibiotic selected from the group consisting of sulbenicillin, mecillinam, carbenicillin, piperacillin, ticarcillin, and thienamycin.

122. Method of claim 114, wherein the anti-biotic is a cephalosporin selected from the group consisting of cefotiam, cefsulodine, cefmenoxime, cefmetazole, cefazolin, cefotaxime, cefoperazone, ceftizoxime and moxalactam.

123. Method of claim 114, wherein the anti-viral bioactive species is acyclovir.

124. A method for treating a cancer in an individual, said method comprising the steps of contacting body tissue of a body cavity of the individual with the suppository of claim 1, wherein the suppository comprises an anti-cancer bioactive substance in an amount effective in treating said cancer.

125. Method of claim 124, wherein the body tissue is a mucosal surface.

126. Method of claim 125, wherein the mucosal surface is a mucosal surface of the rectum.

127. Method of claim 126, wherein the mucosal surface of the rectum is the surface located below the caudal semilunary fold.

128. Method of claim 124, wherein the individual is a human being.

129. Method of claim 124, wherein the anti-cancer bioactive substance is selected from the group consisting of antimetabolites, cytotoxic agents and immunomodulators.

130. Method of claim 129, wherein the antimetabolites are selected from methotrexate, 5-fluorouracil, cytosine arabinoside(ara-C), 5-azacytidine, 6-mercaptopurine, 6-thioguanine, and fludarabine phosphate.

131. Method of claim 129, wherein the cytotoxic agents are selected from taxol, epirubicin, esorubicin, doxorubicin, iodo-doxorubicin, daunorubicin, idarubicin, dactinomycin, bleomycin, mitomycin C, plicamycin, mitoxantrone, vincristine, vinblastine, vindesine, etoposide, and teniposide.

132. Method of claim 124, wherein the anti-cancer bioactive substance is selected from the group consisting of 5'-fluorouracil, mitomycin, cisplatin, taxol, biteomycins, daunomycins, and methamycins.

133. Method for treating a hormone condition in an individual, said method comprising the steps of contacting body tissue of a body cavity of the individual with the suppository of claim 1, wherein the suppository comprises a hormone bioactive substance in an amount effective in treating said deficiency.

134. Method of claim 133, wherein the body tissue is a mucosal surface.

135. Method of claim 134, wherein the mucosal surface is a mucosal surface of the rectum.

136. Method of claim 135, wherein the mucosal surface of the rectum is the surface located below the caudal semilunary fold.

137. Method of claim 133, wherein the individual is a human being.

138. Method of claim 133, wherein the hormone is selected from growth hormone, tPA (tissue plasminogen activator), prolactin, adrenocorticotrophic hormone, melanocyte stimulating hormone, thyrotropin releasing hormone, thyroid stimulating hormone, thyroxine, luteinizing hormone, follicle stimulating hormone, vasopressin, oxytocin, calcitonin, parathyroid hormone, glucagon, gastrin, secretin, pancreozymin, cholecystokinin, angiotensin, human placental lactogen, human chorionic gonadotropin, enkephalin, endorphin, insulin, alpha interferon, beta interferon, and gamma interferon.

139. Method of claim 133, wherein the hormone is selected from corticosteriods, estrogens, progestins, antiestrogens, aromastase inhibitors, androgens, antiandrogens, and endocrines for prostate cancer.

140. Method of claim 133, wherein the hormone is selected from cortisone acetate, hydrocortisone, prednisone, prednisolone, methyl prednisolone and dexamethasone, diethyistibesterol, estradiol, esterified estrogens, conjugated estrogen, chiorotiasnene, medroxyprogesterone acetate, hydroxy progesterone caproate, megestrol acetate, tamoxifen, aminoglutethimide, testosterone propionate, methyltestosterone, fluoxymesterone, testolactone,

flutamide, leuprolide acetate, and ketoconazole.

141. A method for contraception comprising the steps of contacting body tissue of a body cavity of a female mammal with the suppository of claim 1, wherein the suppository comprises a contraceptive bioactive substance in an amount effective to achieve said contraception in said female mammal.

142. Method of claim 141, wherein the body tissue is a mucosal surface.

143. Method of claim 142, wherein the mucosal surface is a mucosal surface of the vagina.

144. Method of claim 141, wherein the mammal is a human being.

145. A surgical method performed on an individual, comprising modifying blood coagulation by performing the steps of contacting body tissue of a body cavity of the individual with the suppository of claim 1, wherein the suppository comprises a coagulation modifying agent in an amount effective to achieve said blood coagulation modification.

146. Method of claim 145, wherein the body tissue is a mucosal surface.

147. Method of claim 146, wherein the mucosal surface is a mucosal surface of the rectum.

148. Method of claim 147, wherein the mucosal surface of the rectum is the surface located below the caudal semilunary fold.

149. Method of claim 145, wherein the individual is a human being.

150. Method for immunising an individual, said method comprising the steps of contacting body tissue of a body cavity of the individual with the suppository of claim 1, wherein the suppository comprises an antigenic substance in an amount effective in achieving said immunisation.

151. Method of claim 150, wherein the body tissue is a mucosal surface.

152. Method of claim 151, wherein the mucosal surface is a mucosal surface of the rectum.

153. Method of claim 152, wherein the mucosal surface of the rectum is the surface located below the caudal semilunary fold.

154. Method of claim 150, wherein the individual is a human being.

155. Method of claim 150, wherein the suppository further comprises an adjuvant.

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		DB=USPT; PLUR=YES; OP=AND	
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END OF SEARCH HISTORY

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L3: Entry 8 of 13 File: USPT Jun 1, 1999

DOCUMENT-IDENTIFIER: US 5908845 A
TITLE: Polyether nucleic acids

Brief Summary Text (3):
An antisense oligonucleotide (e.g., antisense oligodeoxyribonucleotide) may bind its its target nucleic acid either by Watson-Crick base pairing or Hoogsteen and anti-Hoogsteen base pairing. To this effect see, Thuong and Helene (1993) Sequence specific recognition and modification of double helical DNA by oligonucleotides Angev. Chem. Int. Ed. Engl. 32:666. According to the Watson-Crick base pairing, heterocyclic bases of the antisense oligonucleotide form hydrogen bonds with the heterocyclic bases of target single-stranded nucleic acids (RNA or single-stranded DNA), whereas according to the Hoogsteen base pairing, the heterocyclic bases of the target nucleic acid are double-stranded DNA, wherein a third strand is accommodated in the major groove of the B-form DNA duplex by Hoogsteen and anti-Hoogsteen base pairing to form a triplex structure.

Brief Summary Text (4):
According to both the Watson-Crick and the Hoogsteen base pairing models, antisense oligonucleotides have the potential to regulate gene expression and to disrupt the essential functions of the nucleic acids. Therefore, antisense oligonucleotides have possible uses in modulating a wide range of diseases.

Brief Summary Text (7):
Gene expression involves few distinct and well regulated steps. The first major step of gene expression involves transcription of a messenger RNA (mRNA) which is an RNA sequence complementary to the antisense (i.e., -) DNA strand, or, in other words, identical in sequence to the DNA sense (i.e., +) strand, composing the gene. In eukaryotes, transcription occurs in the cell nucleus.

Brief Summary Text (10):
There are also evidence that in some cases gene expression is downregulated by endogenous antisesnse RNA repressors that bind a complementary mRNA transcript and thereby prevent its translation into a functional protein. To this effect see Green et al. (1986) The role of antisense RNA in gene regulation. Ann. Rev. Biochem. 55:569.

Brief Summary Text (17):
At the transcription level, antisense or sense oligonucleotides or analogs that bind bind to the genomic DNA by strand displacement or the formation of a triple helix, may prevent transcription. To this effect see, Thuong and Helene (1993) Sequence specific recognition and modification of double helical DNA by oligonucleotides Angev. Chem. Int. Ed. Engl. 32:666.

Brief Summary Text (18):
At the transcript level, antisense oligonucleotides or analogs that bind target mRNA mRNA molecules lead to the enzymatic cleavage of the hybrid by intracellular RNase H. To this effect see Dash et al. (1987) Proc. Natl. Acad. Sci. U.S.A., 84:7896. In this case, by hybridizing to the targeted mRNA, the oligonucleotides or oligonucleotide analogs provide a duplex hybrid recognized and destroyed by the RNase H enzyme. Alternatively, such hybrid formation may lead to interference with

correct splicing. To this effect see Chiang et al. (1991) Antisense oligonucleotides oligonucleotides inhibit intercellular adhesion molecule 1 expression by two distinct mechanisms. J. Biol. Chem. 266:18162. As a result, in both cases, the number of the target mRNA intact transcripts ready for translation is reduced or eliminated.

Brief Summary Text (19):
At the translation level, antisense oligonucleotides or analogs that bind target mRNA molecules prevent, by steric hindrance, binding of essential translation factors (ribosomes), to the target mRNA, as described by Paterson et al. (1977) Proc. Natl. Acad. Sci. U.S.A., 74:4370, a phenomenon known in the art as hybridization arrest, disabling the translation of such mRNAs.

Brief Summary Text (20):
Thus, antisense sequences, which as described hereinabove may arrest the expression of any endogenous and/or exogenous gene depending on their specific sequence, attracted much attention by scientists and pharmacologists who were devoted at developing the antisense approach into a new pharmacological tool. To this effect see Cohen (1992) Oligonucleotide therapeutics. Trends in Biotechnology, 10:87.

Brief Summary Text (21):
For example, several antisense oligonucleotides have been shown to arrest hematopoietic cell proliferation (Szczylik et al (1991) Selective inhibition of leukemia cell proliferation by BCR-ABL antisense oligodeoxynucleotides. Science 253:562), growth (Calabretta et al. (1991) Normal and leukemic hematopoietic cell manifest differential sensitivity to inhibitory effects of c-myc antisense oligodeoxynucleotides: an in vitro study relevant to bone marrow purging. Proc. Natl. Acad. Sci. U.S.A. 88:2351), entry into the S phase of the cell cycle (Heikhila et al. (1987) A c-myc antisense oligodeoxynucleotide inhibits entry into S S phase but not progress from G(0) to G(1). Nature, 328:445), reduced survival (Reed et al. (1990) Antisense mediated inhibition of BCL2 prooncogene expression and and leukemic cell growth and survival: comparison of phosphodiester and phosphorothioate oligodeoxynucleotides. Cancer Res. 50:6565) and prevent receptor mediated responses (Burch and Mahan (1991) Oligodeoxynucleotides antisense to the interleukin I receptor m RNA block the effects of interleukin I in cultured murine and human fibroblasts and in mice. J. Clin. Invest. 88:1190). For use of antisense oligonucleotides as antiviral agents the reader is referred to Agrawal (1992) Antisense oligonucleotides as antiviral agents. TIBTECH 10:152.

Brief Summary Text (22):
For efficient in vivo inhibition of gene expression using antisense oligonucleotides or analogs, the oligonucleotides or analogs must fulfill the following requirements (i) sufficient specificity in binding to the target sequence; (ii) solubility in water; (iii) stability against intra- and extracellular nucleases; (iv) capability of penetration through the cell membrane; and (v) when used to treat an organism, low toxicity.

Brief Summary Text (23):
Unmodified oligonucleotides are impractical for use as antisense sequences since they have short in vivo half-lives, during which they are degraded rapidly by nucleases. Furthermore, they are difficult to prepare in more than milligram quantities. In addition, such oligonucleotides are poor cell membrane penetraters, see, Uhlmann et al. (1990) Chem. Rev. 90:544.

Brief Summary Text (27):
Oligonucleotides can be modified either in the base, the sugar or the phosphate moiety. These modifications include the use of methylphosphonates, monothiophosphates, dithiophosphates, phosphoramidates, phosphate esters, bridged phosphorothioates, bridged phosphoramidates, bridged methylenephosphonates, dephospho internucleotide analogs with siloxane bridges, carbonate bridges,

carboxymethyl ester bridges, carbonate bridges, carboxymethyl ester bridges, acetamide bridges, carbamate bridges, thioether bridges, sulfoxy bridges, sulfono bridges, various "plastic" DNAs, x-anomeric bridges and borane derivatives. For further details the reader is referred to Cook (1991) Medicinal chemistry of antisense oligonucleotides--future opportunities. Anti-Cancer Drug Design 6:585.

Brief Summary Text (39):
There is thus a widely recognized need for, and it would be highly advantageous to have, oligonucleotide analogs devoid of these drawbacks which are characterized by (i) sufficient specificity in binding to target sequences; (ii) solubility in water; (iii) stability against intra- and extracellular nucleases; (iv) capability of penetrating through cell membranes; and (v) when used to treat an organism, low toxicity, properties that collectively render an oligonucleotide analog highly suitable as an antisense therapeutic drug.

Brief Summary Text (71):
The present invention successfully addresses the shortcomings of the presently known configurations by providing an oligonucleotide analog characterized by (i) sufficient specificity in binding its target sequence; (ii) solubility in water; (iii) stability against intra- and extracellular nucleases; (iv) capability of penetrating through the cell membrane; and (v) when used to treat an organism, low toxicity, properties collectively rendering the oligonucleotide analog of the present invention highly suitable as an antisense therapeutic drug.

Detailed Description Text (2):
The present invention is a compounds that are not polynucleotides yet which bind to complementary DNA and RNA sequences, the compounds according to the invention include naturally occurring necleobases or other nucleobases binding moieties (also referred herein as nucleobase analogs) covalently bound to a polyether backbone, which can be used as oligonucleotide analogs in for example antisense procedures. The oligonucleotide analogs according to the present invention include a new acyclic biopolymer backbone which best fulfills the five criteria for selecting antisense oligonucleotide analogs listed in the background section above.

Detailed Description Text (3):
The synthesis, structure and mode of operation of antisense oligonucleotide analogs according to the present invention may be better understood with reference to the drawings and accompanying descriptions.

Detailed Description Text (40):
The present invention is further directed at therapeutic and/or prophylactic uses for polyether nucleic acids (ENAs). Likely therapeutic and prophylactic targets according to the invention include but are not limited to human papillomavirus (HPV), herpes simplex virus (HSV), candidia albicans, influenza virus, human immunodeficiency virus (HIV), intracellular adhesion molecules (ICAM), cytomegalovirus (CMV), phospholipase A2 (PLA2), 5-lipoxygenase (5-LO), protein kinase C (PKC), and RAS oncogene.

Detailed Description Text (53):
Second, one of the major drawbacks of PNAs when used as antisense molecules is that PNA-DNA hybrids are characterized by high melting temperature (Tm). For example, the Tm value for a duplex such as PNA-T.sub.10 -dA.sub.10 is greater than 70.degree. C., whereas the Tm value of the equivalent native double stranded DNA (dT.sub.10 -dA.sub.10) is nearly three fold lower, about 24.degree. C. Because PNAs bind complementary sequences so strongly, at body temperature (e.g., 37.degree. C.) PNAs lack the specificity to their intended counterparts and end up binding not just to target sequences but also to other strands of DNA, RNA, or even proteins, incapacitating the cell in unforeseen ways. PNAs act as a micelle when the lysine residues are solvated. PNAs are poorly miscible in water, while the hydrophobic nature of the backbone have a tendency to seek for a nonpolar environment e.g., the

bases of the natural complementary DNA. These hydrophobic interactions are the major major driving force for the formation of highly stable PNA-DNA hybrid and therefore very high Tm values for such hybrids. The unique solubility nature of ENAs, by conserving the hydrophobic-hydrophilic properties of polyethers such as PEG, yield Tm values slightly higher than natural DNA, yet much lower values than PNAs, which moderate values are of great importance for specificity.

Detailed Description Text (55):
Fourth, PEG is approved by the FDA for parenteral use, topical application, and as a a constituent of suppositories, nasal sprays, foods and cosmetics. PEG is of low toxicity when administered orally or parenterally, and only large quantifies involve adverse reactions. See, Smyth, H. F. et al. (1955) J. Am. Pharm. Assoc., 34:27. Evidences accumulated experiencing administration of PEG-protein conjugates, suggest that both the plasma half-lives (circulating time) of PEG conjugated proteins and their bioavailability improves as compared with the native proteins, which improvement is accompanied by improved efficacy. Ganser et al. (1989) Blood, 73:31, observed less side effects at lower dosage using PEG-modifications. Reduced toxicity has been observed with several PEG-modified enzymes, see Fuertges et al. (1990) J. Contr. Release, 11:139. Another advantage in exploiting the improved pharmacokinetics of PEG is the option of administrating bolus injections instead of continuous intravenous infusions, as described by Pizzo (1991) Adv. Drug Del. Rev. 6:153. In the preferred embodiments of the invention, ENAs include a PEG backbone and/or are conjugated to PEG exoconjugates and therefore enjoy the above listed advantages.

Detailed Description Text (100):
The dried polymer is then condensed with a second compound following formula V to which a second base (B.sub.2) is attached (e.g., compound H-B.sub.2) in dry DMF in a manner as described above under condensation. Such cycles are repeated as much as needed to form appropriate antisense sequence, wherein in each tri-stages cycle one additional monomer is sequentially added to the growing chain.

Detailed Description Text (103):
The polymeric support to which the antisense sequence is attached, is treated with concentrated ammonium hydroxide for 16 hours at 55.degree. C. The polymeric support is washed with water, methanol and with ether.

Other Reference Publication (1):
Burch et al, "Oligonucleotides Antisense to the Interlukin 1 Receptor mRNA Block the the Effects of Interlukin 1 in Cultures Murine and Human Fibroblasts and in Mice", J. Clin. Inv., vol. 88, pp. 1190-1196, (1991).

Other Reference Publication (2):
Calabretta et al, "Normal and leukemic hematopoietic cells manifest differential sensitivity to inhibitory effects of c-myb antisense ologideoxynucleotides: An in vitro study relevant to bone marrow purging", Proc. Natl. Acad. Sci. USA, vol. 88, pp. 2351-2355, (1991).

Other Reference Publication (4):
Heikkila et al, "A c-myb antisense ologideoxynucleotide inhibits entry into S phase but not progress from G.sub.0 to G.sub.1 ", Nature, vol. 328, 445-449, (1987).

Other Reference Publication (8):
Szczylik et al, "Selective Inhibition of Leukemia Cell Proliferation by BCR-ABL Antisense Ologideoxynucleotides", Science, vol. 253, pp. 562-565, (1991).

Other Reference Publication (9):
Uhlmann et al, "Antisense Ologideoxynucleotides: A New Therepeutic Principle", Chem. Chem. Rev., vol. 90, No. 4, pp. 544-584, (1990).

Other Reference Publication (17):
Agrawal, S., "Antisense ologideoxynucleotides as antiviral agents", Tibtech, vol. 10, No. 5, pp. 152-158, (1992).

Other Reference Publication (18):
Wahlestedt, C., "Antisense ologideoxynucleotide strategies in neuropharmacology", TIPS, vol. 15, pp. 42-46, (1994).

Other Reference Publication (19):
Cook, P.D., "Medicinal chemistry of antisense ologideoxynucleotides -- future opportunities", Anti-Cancer Drug design, vol. 6, pp. 585-607, (1991).

Other Reference Publication (20):
Green, et al, "The Role of Antisense RNA in Gene regulation", Ann. Rev. Biochem., vol. 55, pp. 569-597, (1986).

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L19: Entry 11 of 16 File: USPT Oct 23, 2001

DOCUMENT-IDENTIFIER: US 6306993 B1
TITLE: Method and composition for enhancing transport across biological membranes

Brief Summary Text (32):
Frankel et al. (1991) reported that conjugating selected molecules to the tat protein of HIV can increase cellular uptake of those molecules. However, use of the tat protein has certain disadvantages, including unfavorable aggregation and insolubility properties.

Brief Summary Text (35):
The present invention is based in part on the applicants' discovery that conjugation of certain polymers composed of contiguous, highly basic subunits, particularly subunits containing guanidyl or amidinyl moieties, to small molecules or macromolecules is effective to significantly enhance transport of the attached molecule across biological membranes. Moreover, transport occurs at a rate significantly greater than the transport rate provided by a basic HIV tat peptide consisting of residues 49-57.

Brief Summary Text (46):
Biologically active agents (which encompass therapeutic agents) include, but are not limited to metal ions, which are typically delivered as metal chelates; small organic molecules, such as anticancer (e.g., taxane) and antimicrobial molecules (e.g., against bacteria or fungi such as yeast); and macromolecules such as nucleic acids, peptides, proteins, and analogs thereof. In one preferred embodiment, the agent is a nucleic acid or nucleic acid analog, such as a ribozyme which optionally contains one or more 2'-deoxy nucleotide subunits for enhanced stability. Alternatively, the agent is a peptide nucleic acid (PNA). In another preferred embodiment, the agent is a polypeptide, such as a protein antigen, and the biological membrane is a cell membrane of an antigen-presenting cell (APC). In another embodiment, the agent is selected to promote or elicit an immune response against a selected tumor antigen. In another preferred embodiment, the agent is a taxane or taxoid anticancer compound. In another embodiment, the agent is a non-polypeptide agent, preferably a non-polypeptide therapeutic agent. In a more general embodiment, the agent preferably has a molecular weight less than 10 kDa.

Detailed Description Text (62):
FIGS. 1A-1C show results from a study in which polymers of L-arginine (R; FIG. 1A) or D-arginine (r; FIG. 1B) ranging in length from 4 to 9 arginine subunits were tested for ability to transport fluorescein into Jurkat cells. For comparison, transport levels for an HIV tat residues 49-57 ("49-57") and a nonamer of L-lysine (K9) were also tested. FIG. 1C shows a histogram of uptake levels for the conjugates at a concentration of 12.5 .mu.M.

Detailed Description Text (66):
From the foregoing, it is apparent that transport polymers of the invention are significantly more effective than HIV tat peptide 47-59 in transporting drugs across across the plasma membranes of cells. Moreover, the poly-Lys nonamer was ineffective as a transporter.

Detailed Description Text (75):
Experiments carried out in support of the present invention indicate that polymer-facilitated transport is dependent upon metabolic integrity of cells. Addition of a toxic amount of sodium azide (0.5% w/v) to cells resulted in inhibition of uptake of conjugates by about 9% (Example 7). The results shown in FIG. 4 demonstrate (i) sodium azide sensitivity of trans-membrane transport, suggesting energy-dependence (cellular uptake), and (ii) the superiority of poly-guanidinium polymers of the invention (R9, R8, R7) relative to HIV tat(49-57).

Detailed Description Text (104):
Examples of anti-sense oligonucleotides whose transport into cells may be enhanced using the methods of the invention are described, for example, in U.S. Pat. No. 5,594,122. Such oligonucleotides are targeted to treat human immunodeficiency virus (HIV). Conjugation of a transport polymer to an anti-sense oligonucleotide can be effected, for example, by forming an amide linkage between the peptide and the 5'-terminus of the oligonucleotide through a succinate linker, according to well-established methods. The use of PNA conjugates is further illustrated in Example 11.

Detailed Description Text (107):
In addition, and according to an important aspect of the invention, protein antigens may be delivered to the cytosolic compartment of antigen-presenting cells (APCs), where they are degraded into peptides. The peptides are then transported into the endoplasmic reticulum, where they associate with nascent HLA class I molecules and are displayed on the cell surface. Such "activated" APCs can serve as inducers of class I restricted antigen-specific cytotoxic T-lymphocytes (CTLs), which then proceed to recognize and destroy cells displaying the particular antigen. APCs that are able to carry out this process include, but are not limited to, certain macrophages, B cells and dendritic cells. In one embodiment, the protein antigen is a tumor antigen for eliciting or promoting an immune response against tumor cells.

Detailed Description Text (108):
The transport of isolated or soluble proteins into the cytosol of APC with subsequent activation of CTL is exceptional, since, with few exceptions, injection of isolated or soluble proteins does not result either in activation of APC or induction of CTLs. Thus, antigens that are conjugated to the transport enhancing compositions of the present invention may serve to stimulate a cellular immune response in vitro or in vivo.

Detailed Description Text (109):
Example 14 provides details of experiments carried out in support of the present invention in which an exemplary protein antigen, ovalbumin, was delivered to APCs after conjugation to an R7 polymer. Subsequent addition of the APCs to cytotoxic T lymphocytes (CTLs) resulted in CD8+ albumin-specific cytotoxic T cells (stimulated CTLs). In contrast, APCs that had been exposed to unmodified ovalbumin failed to stimulate the CTLs.

Detailed Description Text (111):
In another embodiment, the invention is useful for delivering immunospecific antibodies or antibody fragments to the cytosol to interfere with deleterious biological processes such as microbial infection. Recent experiments have shown that intracellular antibodies can be effective antiviral agents in plant and mammalian cells (e.g., Tavladoraki et al., 1993; and Shaheen et al., 1996). These methods have typically used single-chain variable region fragments (scFv), in which the antibody heavy and light chains are synthesized as a single polypeptide. The variable heavy and light chains are usually separated by a flexible linker peptide (e.g., of 15 amino acids) to yield a 28 kDa molecule that retains the high affinity ligand binding site. The principal obstacle to wide application of this technology has been efficiency of uptake into infected cells. But by attaching transport

polymers to scFv fragments, the degree of cellular uptake can be increased, allowing allowing the immunospecific fragments to bind and disable important microbial components, such as HIV Rev, HIV reverse transcriptase, and integrase proteins.

Detailed Description Text (113):
Peptides to be delivered by the enhanced transport methods described herein include, but should not be limited to, effector polypeptides, receptor fragments, and the like. Examples include peptides having phosphorylation sites used by proteins mediating intracellular signals. Examples of such proteins include, but are not limited to, protein kinase C, RAF-1, p21Ras, NF-.kappa.B, C-JUN, and cytoplasmic tails of membrane receptors such as IL-4 receptor, CD28, CTLA-4, V7, and MHC Class I and Class II antigens.

Detailed Description Text (133):
The active compounds of the formulas may be formulated into a suppository comprising, for example, about 0.5% to about 50% of a compound of the invention, disposed in a polyethylene glycol (PEG) carrier (e.g., PEG 1000 [96%] and PEG 4000 [4%]).

Detailed Description Text (150):
Uptake levels of the following polypeptides were measured by the method in Example 2: (1) a polypeptide comprising HIV tat residues 49-57 (Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Arg=SEQ ID NO:1), (2) a nonamer of L-Lys residues (K9, SEQ ID NO:2), and (3) homo-L or homo-D polypeptides containing four to nine Arg residues (SEQ ID NO:3-8 and 12-17). Results a re shown in FIGS. 2A-2C.

Detailed Description Text (207):
3. Gamma-IFN Assay. The amount of gamma interferon secreted by a murine T cell line (clone 11.3) was measured by incubating 10.sup.5 T cells with varying amounts of antigen (peptide consisting of residues 110-121 of sperm whale myoglobin) and histocompatible spleen cells from DBA/2 mice (H-2d, 5.times.10.sup.5), which act as antigen-presenting cells (APCs), in 96 well plates. After incubation for 24 hours at at 37.degree. C., 100 .mu.L of the supernatants were transferred to microtiter plates coated with commercially available anti-gamma-IFN monoclonal antibodies (Mab) (Pharmingen, San Diego, Calif.). After incubation for an hour at room temperature, the plates were washed with PBS containing 1% fetal calf serum and 0.1% Tween 20, after which a second, biotinylated gamma-IFN Mab was added. After a second hour of incubation, the plates were washed as before, and europium (Eu)-streptavidin (Delphia-Pharmacia) was added. Again, after an hour of incubation, an acidic buffer was added to release Eu, which was measured by time-resolved fluorometry on a Delphia plate reader. The amount of fluorescence was proportional to the amount of gamma-IFN that had been produced and could be quantified precisely using known amounts of gamma-IFN to create a standard curve.

Detailed Description Text (208):
4. Inhibition of Gamma-IFN Production by Conjugates. The ability of PNA-polyarginine conjugates to inhibit secretion of gamma-IFN was assayed by adding various concentrations of the above gamma-IFN conjugates with suboptimal doses of peptide antigen (0.5 AM), to a mixture of clone 11.3 T cells and histocompatible spleen cells. PNA sequences lacking polyarginine moieties, and non-conjugated D-arginine heptamer, were also tested.

Detailed Description Text (211):
Transport of Large Protein Antigen Into APCs

Detailed Description Text (212):
A conjugate of ovalbumin coupled to a poly-L-arginine heptamer was formed by reacting a cysteine-containing polypeptide polymer (Cys-Ala-Ala-Ala-Arg.sub.7, SEQ ID NO:20) with ovalbumin (45 kDa) in the presence of sulfo-MBS, a heterobifunctional crosslinker (Pierce Chemical Co., Rockford, Ill.). The molar

ratio of peptide conjugated to ovalbumin was quantified by amino acid analysis. The conjugate product was designated OV-R7. The conjugate was added (final concentration .infin.10 .mu.M) to B-cells, also referred to as antigen-presenting cells (APCs), which were isolated according to standard methods. The APCs were incubated with OV-R7, and then were added to a preparation of cytotoxic T-lymphocytes isolated by standard methods. Exposure of CTLs to APCs that had been incubated with OV-R7 produced CD8+ albumin-specific CTLs. In contrast, APCs that had been exposed to unmodified ovalbumin failed to stimulate the CTLs.

Other Reference Publication (15):
Buschle, M. et al., "Transloading of tumor antigen-derived peptides in antigen-presenting cells," Proc. Natl. Acad. Sci. 94:3256-3261 (1997).

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L18: Entry 22 of 102 File: USPT Oct 23, 2001

DOCUMENT-IDENTIFIER: US 6306993 B1
TITLE: Method and composition for enhancing transport across biological membranes

Brief Summary Text (46):
Biologically active agents (which encompass therapeutic agents) include, but are not limited to metal ions, which are typically delivered as metal chelates; small organic molecules, such as anticancer (e.g., taxane) and antimicrobial molecules (e.g., against bacteria or fungi such as yeast); and macromolecules such as nucleic acids, peptides, proteins, and analogs thereof. In one preferred embodiment, the agent is a nucleic acid or nucleic acid analog, such as a ribozyme which optionally contains one or more 2'-deoxy nucleotide subunits for enhanced stability. Alternatively, the agent is a peptide nucleic acid (PNA). In another preferred embodiment, the agent is a polypeptide, such as a protein antigen, and the biological membrane is a cell membrane of an antigen-presenting cell (APC). In another embodiment, the agent is selected to promote or elicit an immune response against a selected tumor antigen. In another preferred embodiment, the agent is a taxane or taxoid anticancer compound. In another embodiment, the agent is a non-polypeptide agent, preferably a non-polypeptide therapeutic agent. In a more general embodiment, the agent preferably has a molecular weight less than 10 kDa.

Detailed Description Text (107):
In addition, and according to an important aspect of the invention, protein antigens may be delivered to the cytosolic compartment of antigen-presenting cells (APCs), where they are degraded into peptides. The peptides are then transported into the endoplasmic reticulum, where they associate with nascent HLA class I molecules and are displayed on the cell surface. Such "activated" APCs can serve as inducers of class I restricted antigen-specific cytotoxic T-lymphocytes (CTLs), which then proceed to recognize and destroy cells displaying the particular antigen. APCs that are able to carry out this process include, but are not limited to, certain macrophages, B cells and dendritic cells. In one embodiment, the protein antigen is a tumor antigen for eliciting or promoting an immune response against tumor cells.

Detailed Description Text (108):
The transport of isolated or soluble proteins into the cytosol of APC with subsequent activation of CTL is exceptional, since, with few exceptions, injection of isolated or soluble proteins does not result either in activation of APC or induction of CTLs. Thus, antigens that are conjugated to the transport enhancing compositions of the present invention may serve to stimulate a cellular immune response in vitro or in vivo.

Detailed Description Text (109):
Example 14 provides details of experiments carried out in support of the present invention in which an exemplary protein antigen, ovalbumin, was delivered to APCs after conjugation to an R7 polymer. Subsequent addition of the APCs to cytotoxic T lymphocytes (CTLs) resulted in CD8+ albumin-specific cytotoxic T cells (stimulated CTLs). In contrast, APCs that had been exposed to unmodified ovalbumin failed to stimulate the CTLs.

Detailed Description Text (113):

Peptides to be delivered by the enhanced transport methods described herein include, include, but should not be limited to, effector polypeptides, receptor fragments, and the like. Examples include peptides having phosphorylation sites used by proteins mediating intracellular signals. Examples of such proteins include, but are are not limited to, protein kinase C, RAF-1, p21Ras, NF-.kappa.B, C-JUN, and cytoplasmic tails of membrane receptors such as IL-4 receptor, CD28, CTLA-4, V7, and and MHC Class I and Class II antigens.

Detailed Description Text (133):
The active compounds of the formulas may be formulated into a suppository comprising, for example, about 0.5% to about 50% of a compound of the invention, disposed in a polyethylene glycol (PEG) carrier (e.g., PEG 1000 [96%] and PEG 4000 [4%]).

Detailed Description Text (207):
3. Gamma-IFN Assay. The amount of gamma interferon secreted by a murine T cell line (clone 11.3) was measured by incubating 10.sup.5 T cells with varying amounts of antigen (peptide consisting of residues 110-121 of sperm whale myoglobin) and histocompatible spleen cells from DBA/2 mice (H-2d, 5.times.10.sup.5), which act as antigen-presenting cells (APCs), in 96 well plates. After incubation for 24 hours at at 37.degree. C., 100 .mu.L of the supernatants were transferred to microtiter plates coated with commercially available anti-gamma-IFN monoclonal antibodies (Mab) (Pharmingen, San Diego, Calif.). After incubation for an hour at room temperature, the plates were washed with PBS containing 1% fetal calf serum and 0.1% Tween 20, after which a second, biotinylated gamma-IFN Mab was added. After a second hour of incubation, the plates were washed as before, and europium (Eu)-streptavidin (Delphia-Pharmacia) was added. Again, after an hour of incubation, an acidic buffer was added to release Eu, which was measured by time-resolved fluorometry on a Delphia plate reader. The amount of fluorescence was proportional to the amount of gamma-IFN that had been produced and could be quantified precisely using known amounts of gamma-IFN to create a standard curve.

Detailed Description Text (208):
4. Inhibition of Gamma-IFN Production by Conjugates. The ability of PNA-polyarginine conjugates to inhibit secretion of gamma-IFN was assayed by adding various concentrations of the above gamma-IFN conjugates with suboptimal doses of peptide antigen (0.5 AM), to a mixture of clone 11.3 T cells and histocompatible spleen cells. PNA sequences lacking polyarginine moieties, and non-conjugated D-arginine heptamer, were also tested.

Detailed Description Text (211):
Transport of Large Protein Antigen Into APCs

Detailed Description Text (212):
A conjugate of ovalbumin coupled to a poly-L-arginine heptamer was formed by reacting a cysteine-containing polypeptide polymer (Cys-Ala-Ala-Ala-Arg.sub.7, SEQ ID NO:20) with ovalbumin (45 kDa) in the presence of sulfo-MBS, a heterobifunctional crosslinker (Pierce Chemical Co., Rockford, Ill.). The molar ratio of peptide conjugated to ovalbumin was quantified by amino acid analysis. The conjugate product was designated OV-R7. The conjugate was added (final concentration .infin.10 .mu.M) to B-cells, also referred to as antigen-presenting cells (APCs), which were isolated according to standard methods. The APCs were incubated with OV-R7, and then were added to a preparation of cytotoxic T-lymphocytes isolated by standard methods. Exposure of CTLs to APCs that had been incubated with OV-R7 produced CD8+ albumin-specific CTLs. In contrast, APCs that had been exposed to unmodified ovalbumin failed to stimulate the CTLs.

Other Reference Publication (15):
Buschle, M. et al., "Transloading of tumor antigen-derived peptides in antigen-presenting cells," Proc. Natl. Acad. Sci. 94:3256-3261 (1997).

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L8: Entry 2 of 4 File: USPT Mar 13, 2001

US-PAT-NO: 6200590
DOCUMENT-IDENTIFIER: US 6200590 B1

TITLE: Controlled, phased-release suppository and its method of production

DATE-ISSUED: March 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Eley; John Graham	Alabaster	AL		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Naphcare, Inc.	Birmingham	AL			02

APPL-NO: 09/ 131591 [PALM]
DATE FILED: August 10, 1998

INT-CL: [07] A01 N 43/22

US-CL-ISSUED: 424/433; 424/434, 424/DIG.15, 514/965
US-CL-CURRENT: 424/433; 424/434, 424/DIG.15, 514/965

FIELD-OF-SEARCH: 424/433, 424/434, 424/DIG.15, 514/965

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4652441	March 1987	Okada et al.	424/19
☐	5215758	June 1993	Krishnamurthy	424/488
☐	5500221	March 1996	Murata et al.	424/436
☐	5518730	May 1996	Fuisz	424/426
☐	5985313	November 1999	Neurath et al.	424/434

OTHER PUBLICATIONS

E. Allemann, et al., "International Journal of Pharmaceutics", vol. 87, pp. 247-253 (1992).

ART-UNIT: 165

PRIMARY-EXAMINER: Azpuru; Carlos A.

ATTY-AGENT-FIRM: May; Wm. Randall

ABSTRACT:

A phased-release suppository delivery system is disclosed wherein microscopic polymeric "nanospheres" ladened with one or more active agents are homogeneously incorporated within a pharmaceutically acceptable suppository base. The preparation of the "nanospheres" allows the spheres to be transported, substantially intact, across fenestrated membranes such as the capillary membranes of the rectum. The method of preparation of the "nanospheres" allows for the controlled release of active agent(s) only after a substantial number of the spheres have been transported transported across the capillary membrane of the rectum or other body cavity and have been taken up into the systemic circulation system.

8 Claims, 5 Drawing figures

L8: Entry 2 of 4 File: USPT Mar 13, 2001

DOCUMENT-IDENTIFIER: US 6200590 B1
TITLE: Controlled, phased-release suppository and its method of production

CLAIMS:

1. A four-stage, phased-release drug delivery means, comprising a suppository preparation comprising drug-ladened, polymeric synthesized microscopic particles within a pharmaceutically acceptable suppository base component wherein said microscopic particles have been specially formulated as a homogeneous matrix of active agent(s) and biodegradable polymers wherein the process for formulating said microscopic particles produces drug-ladened spheres no larger than 300 nanometers in diameter, the size and formulation of which allows these "nanoparticles" to be delivered, substantially intact, across the fenestrated capillaries of the rectal or vaginal mucosa for the controlled release of active agent(s) directly into the subject's systemic circulation; said four-stage, phased-release, delivery means comprising first, the delivery of the suppository containing said drug-ladened nanoparticle spheres into the rectal or vaginal cavity; second, the aquaous solubility of the chosen suppository base component; third, the uptake of said nanoparticle spheres into the fenestrated capillaries of the chosen cavity; and fourth, the release of active agent(s), controlled by the percentage composition of polymers used in the production of the nanoparticles, directly into the subject's systemic circulation.

6. The drug delivery means of claim 1, wherein the suppository base component comprises a pharmaceutically acceptable form of polyethylene glycol.

7. The drug delivery means of claim 1, wherein the suppository base component is chosen from the group consisting of cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, fatty acid esters of polyethylene glycols, glycolsurfactant PEGs, glycerinated gelatin, and nonionic surfactant materials such as polyoxyethylene derivatives of sorbitan monostearate and polyoxyl--40 stearate.

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L3: Entry 2 of 13 File: USPT Dec 30, 2003

DOCUMENT-IDENTIFIER: US 6669951 B2
TITLE: Compositions and methods for enhancing drug delivery across and into epithelial tissues

Detailed Description Text (14):
The terms "non-polypeptide agent" and "non-polypeptide therapeutic agent" refer to the portion of a conjugate that does not include the delivery-enhancing transporter, and that is a biologically active agent other than a polypeptide. An example of a non-polypeptide agent is an anti-sense oligonucleotide, which can be conjugated to a poly-arginine peptide to form a conjugate for enhanced delivery into and across one or more layers of an epithelial or endothelial tissue.

Detailed Description Text (23):
The delivery-enhancing transporters increase delivery of the conjugate into and across one or more intact epithelial or endothelial tissue layers compared to delivery of the compound in the absence of the delivery-enhancing transporter. The delivery-enhancing transporters can, in some embodiments, increase delivery of the conjugate significantly over that obtained using the tat protein of HIV-1 (Frankel et al. (1991) PCT Pub. No. WO 91/09958). Delivery is also increased significantly over the use of shorter fragments of the tat protein containing the tat basic region (residues 49-57 having the sequence RKKKRRQRRR; SEQ ID NO:28) (Barsoum et al. (1994) WO 94/04686 and Fawell et al. (1994) Proc. Nat'l. Acad. Sci. USA 91: 664-668). Preferably, delivery obtained using the transporters of the invention is increased more than 2-fold, still more preferably six-fold, still more preferably ten-fold, and still more preferably twenty-fold, over that obtained with tat residues 49-57.

Detailed Description Text (115):
The active compounds of the formulas may be formulated into a suppository comprising, for example, about 0.5% to about 50% of a compound of the invention, disposed in a polyethylene glycol (PEG) carrier (e.g., PEG 1000 [96%] and PEG 4000 [4%]).

Detailed Description Text (175):
The enhanced transport methods of the invention are particularly suited for enhancing transport into and across one or more layers of an epithelial or endothelial tissue for a number of macromolecules, including, but not limited to proteins, nucleic acids, polysaccharides, and analogs thereof. Exemplary nucleic acids include oligonucleotides and polynucleotides formed of DNA and RNA, and analogs thereof, which have selected sequences designed for hybridization to complementary targets (e.g., antisense sequences for single- or double-stranded targets), or for expressing nucleic acid transcripts or proteins encoded by the sequences. Analogs include charged and preferably uncharged backbone analogs, such as phosphonates (preferably methyl phosphonates), phosphoramidates (N3' or N5'), thiophosphates, uncharged morpholino-based polymers, and protein nucleic acids (PNAs). Such molecules can be used in a variety of therapeutic regimens, including enzyme replacement therapy, gene therapy, and anti-sense therapy, for example.

Detailed Description Text (176):

By way of example, protein nucleic acids (PNA) are analogs of DNA in which the backbone is structurally homomorphous with a deoxyribose backbone. The backbone consists of N-(2-aminoethyl)glycine units to which the nucleobases are attached. PNAs containing all four natural nucleobases hybridize to complementary oligonucleotides obeying Watson-Crick base-pairing rules, and is a true DNA mimic in in terms of base pair recognition (Egholm et al. (1993) Nature 365:566-568. The backbone of a PNA is formed by peptide bonds rather than phosphate esters, making it it well-suited for anti-sense applications. Since the backbone is uncharged, PNA/DNA PNA/DNA or PNA/RNA duplexes that form exhibit greater than normal thermal stability. PNAs have the additional advantage that they are not recognized by nucleases or proteases. In addition, PNAs can be synthesized on an automated peptides synthesizer using standard t-Boc chemistry. The PNA is then readily linked to a transport polymer of the invention.

Detailed Description Text (177):
Examples of anti-sense oligonucleotides whose transport into and across epithelial and endothelial tissues can be enhanced using the methods of the invention are described, for example, in U.S. Pat. No. 5,594,122. Such oligonucleotides are targeted to treat human immunodeficiency virus (HIV). Conjugation of a transport polymer to an anti-sense oligonucleotide can be effected, for example, by forming an an amide linkage between the peptide and the 5'-terminus of the oligonucleotide through a succinate linker, according to well-established methods. The use of PNA conjugates is further illustrated in Example 11 of PCT Application PCT/US98/10571. FIG. 7 of that application shows results obtained with a conjugate of the invention containing a PNA sequence for inhibiting secretion of gamma-interferon (.gamma.-IFN) by T cells, as detailed in Example 11. As can be seen, the anti-sense PNA conjugate was effective to block .gamma.-IFN secretion when the conjugate was present at levels above about 10 .mu.M. In contrast, no inhibition was seen with the sense-PNA conjugate or the non-conjugated antisense PNA alone.

Detailed Description Text (180):
In another embodiment, the invention is useful for delivering immunospecific antibodies or antibody fragments to the cytosol to interfere with deleterious biological processes such as microbial infection. Recent experiments have shown that intracellular antibodies can be effective antiviral agents in plant and mammalian cells (e.g., Tavladoraki et al. (1993) Nature 366:469; and Shaheen et al. (1996) J. Virol. 70:3392. These methods have typically used single-chain variable region fragments (scFv), in which the antibody heavy and light chains are synthesized as a single polypeptide. The variable heavy and light chains are usually separated by a flexible linker peptide (e.g., of 15 amino acids) to yield a 28 kDa molecule that retains the high affinity ligand binding site. The principal obstacle to wide application of this technology has been efficiency of uptake into infected cells. But by attaching transport polymers to scFv fragments, the degree of cellular uptake can be increased, allowing the immunospecific fragments to bind and disable important microbial components, such as HIV Rev, HIV reverse transcriptase, and integrase proteins.

Detailed Description Paragraph Table (2):
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L18: Entry 5 of 102 File: USPT Dec 30, 2003

US-PAT-NO: 6669951
DOCUMENT-IDENTIFIER: US 6669951 B2

TITLE: Compositions and methods for enhancing drug delivery across and into epithelial tissues

DATE-ISSUED: December 30, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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APPL-NO: 09/ 792480 [PALM]
DATE FILED: February 23, 2001

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation-in-part application of U.S. patent application Ser. No. 09/648,400, Aug. 24, 2000, now U.S. Pat. No. 6,593,292, which claims priority to U.S. Provisional Patent Application No. 60/150,510, filed Aug. 24, 1999. Both of these applications are incorporated herein by reference for all purposes.

INT-CL: [07] A61 K 9/02, A61 K 38/03, A61 K 38/08, A61 K 38/13, A61 K 47/42

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US-CL-CURRENT: 424/436; 514/11, 514/16, 514/169, 514/2, 514/634, 514/636, 530/300, 530/329, 564/236, 564/243

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PRIOR-ART-DISCLOSED:

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ART-UNIT: 1654

PRIMARY-EXAMINER: Russel; Jeffrey E.

ATTY-AGENT-FIRM: Townsend and Townsend and Crew LLP

ABSTRACT:

This invention provides compositions and methods for enhancing delivery of drugs and other agents across epithelial tissues, including the skin, gastrointestinal tract, pulmonary epithelium, ocular tissues and the like. The compositions and methods are also useful for delivery across endothelial tissues, including the blood brain barrier. The compositions and methods employ a delivery enhancing transporter that has sufficient guanidino or amidino sidechain moieties to enhance delivery of a compound conjugated to the reagent across one or more layers of the tissue, compared to the non-conjugated compound. The delivery-enhancing polymers include, for example, poly-arginine molecules that are preferably between about 6 and 25 residues in length.

88 Claims, 51 Drawing figures

WEST Search History

Hide Items | Restore | Clear | Cancel

Updated Search 6/04/04

DATE: Monday, June 14, 2004

Hide?	Set Name	Query	Hit Count
		DB=USPT; PLUR=YES; OP=AND	
☐	L1	(6451339 or 6383471 or 6340479 or 6309663 or 6267985 or 5853767).pn.	6
☐	L2	L1 and (polyethylene or peg or poly-ethylene or glycol)	6
☐	L3	L2 and supposit$	6
☐	L4	L1 and supposit$	6
☐	L5	('6383471' |'6340479' |'6451339' |'5853767' |'6267985' |'6309663')!.PN.	6
☐	L6	supposit$.clm.	1342
☐	L7	L6 and peg.clm.	8
☐	L8	L7 not l5	4
☐	L9	l6 and (peg$ or polyethyleneglycol or poly-ethylene-glycol or poly-glycol).clm.	15
☐	L10	L9 nto l7	0
☐	L11	L9 not l7	7
☐	L12	('6200590' |'6610317' |'6284269' |'4686215' |'6645528' |'6589557' |'5972333' |'6437143')!.PN.	8
☐	L13	l6 and (vaccin$ or viral or virus or microbial or microorganism or micro-organism or pathogen or pathogenic)	483
☐	L14	l6 and (vaccin$ or viral or virus or microbial or microorganism or micro-organism or pathogen or pathogenic).clm.	107
☐	L15	l6 and (viral or virus or microbial or microorganism or micro-organism or pathogen or pathogenic).clm.	102
☐	L16	l6 and antigen.clm.	9
☐	L17	suppositor$ same (peg$ or peg or polyethyleneglycol or poly-glycol or poly-ethylene-glycol)	935
☐	L18	L17 and (antigen or antigenic or immunogen or immuno-gen or immunogenic)	102
☐	L19	l18 and hiv	16

END OF SEARCH HISTORY

First Hit Fwd Refs

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L8: Entry 4 of 4 File: USPT Sep 14, 1993

DOCUMENT-IDENTIFIER: US 5244668 A
TITLE: Low-melting moldable pharmaceutical excipient and dosage forms prepared therewith

CLAIMS:

2. The composition of claim 1 wherein said low MW polyethylene glycol is PEG 1000.

3. The composition of claim 1 wherein said medium to high MW polyethylene glycol is PEG 8000.

5. The composition of claim 1 wherein said medium to high molecular weight PEG is present in a proportion of about 0.5% by weight.

6. The composition of claim 5 wherein said medium to high molecular weight PEG is PEG 8000.

12. The dosage form of claim 11 wherein said low MW polyethylene glycol is PEG 1000.

13. The dosage form of claim 11 wherein said medium to high MW polyethylene glycol is PEG 8000.

15. The dosage form of claim 11 wherein said low molecular weight polyethylene glycol is PEG 1000.

16. The dosage form of claim 11 wherein said medium to high molecular weight PEG is present in a proportion of about 0.5% by weight.

17. The dosage form of claim 11 wherein said medium to high molecular weight PEG is PEG 8000.

24. The dosage form of claim 11 wherein said dosage form is a suppository.

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L8: Entry 4 of 4 File: USPT Sep 14, 1993

US-PAT-NO: 5244668
DOCUMENT-IDENTIFIER: US 5244668 A

TITLE: Low-melting moldable pharmaceutical excipient and dosage forms prepared therewith

DATE-ISSUED: September 14, 1993

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Snipes; Wallace C.	Pine Grove Mills	PA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Zetachron, Inc.	State College	PA			02

APPL-NO: 07/ 930325 [PALM]
DATE FILED: August 17, 1992

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATION This is a divisional of copending application(s) Ser. No. 07/677,573, filed on Mar. 29, 1991, U.S. Pat. No. 5,139,790, which is a division of application Ser. No. 07/264,747, filed Oct. 31, 1988, now U.S. Pat. No. 5,004,601, which is a continuation in part of application Ser. No. 07/257,569, filed Oct. 14, 1988, now U.S. Pat. No. 5,135,752.

INT-CL: [05] A61K 9/02, A61K 9/06, A61K 9/20, A61K 31/74

US-CL-ISSUED: 424/435; 424/436, 424/452, 424/465, 424/486, 424/501, 514/770, 514/784, 514/813, 514/874, 514/960, 514/966, 514/967, 514/969, 514/953
US-CL-CURRENT: 424/435; 424/436, 424/452, 424/465, 424/486, 424/501, 514/770, 514/784, 514/813, 514/874, 514/953, 514/960, 514/966, 514/967, 514/969

FIELD-OF-SEARCH: 424/435, 424/436, 424/452, 424/465, 424/486, 424/501, 514/770, 514/960, 514/967, 514/966, 514/953

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL | Clear

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL

☐	3873485	March 1975	Fichera	524/612
☐	4911859	March 1990	Bunczk et al.	252/106
☐	5004601	April 1991	Snipes	514/772.7
☐	5019311	May 1991	Koslow	264/122
☐	5043090	August 1991	Camp et al.	252/106
☐	5082655	January 1992	Snipes et al.	424/486
☐	5133892	July 1992	Chun et al.	252/90
☐	5135752	August 1992	Snipes	424/435
☐	5139790	August 1992	Snipes	424/435
☐	5147722	September 1992	Koslow	264/122

ART-UNIT: 125

PRIMARY-EXAMINER: Rose; Shep K.

ATTY-AGENT-FIRM: Vorys, Sater, Seymour & Pease

ABSTRACT:

An excipient for a pharmaceutical compound which melts at body temperature but will not spontaneously deform at higher temperatures encountered in shipment and storage comprises:

_____ Low MW Polyethylene glycol 75-90% (M.P. about about 37.degree. C.) Medium to high MW polyethylene glycol 0-4% Long chain saturated saturated carboxylic acid 0-4% Polyethylene oxide 0-4% (MW 100,000-5,000,000) Colloidal silica 10-20%. _____

The excipient is particularly advantageous for the preparation of dosage forms for buccal administration of pharmaceutical compounds.

27 Claims, 0 Drawing figures

First Hit Fwd Refs

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L8: Entry 3 of 4 File: USPT Jan 14, 1997

US-PAT-NO: 5593691
DOCUMENT-IDENTIFIER: US 5593691 A

TITLE: Biotenside solvents for pharmaceuticals and cosmetics

DATE-ISSUED: January 14, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Eugster; Carl	Riehen			CH
Eugster; Conrad H.	Wallisellen			CH
Haldemann; Walter	Binningen			CH
Rivara; Giorgio	Turin			IT

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Marigen S.A.	Riehen			CH	03

APPL-NO: 08/ 179729 [PALM]
DATE FILED: January 11, 1994

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
CH	1882/92	June 3, 1993

INT-CL: [06] A61 K 9/62

US-CL-ISSUED: 424/461; 424/401, 424/436, 424/451, 424/459, 424/463, 424/474, 424/489, 549/546, 560/201, 560/220, 560/225
US-CL-CURRENT: 424/461; 424/401, 424/436, 424/451, 424/459, 424/463, 424/474, 424/489, 549/546, 560/201, 560/220, 560/225

FIELD-OF-SEARCH: 424/401, 424/451, 424/459, 424/461, 424/474, 424/489, 424/436, 560/201, 560/220, 560/225, 549/546

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	3480663	November 1969	Thiele	260/482

4256600	March 1981	Lewis et al.	252/132

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0681891	June 1993	CH	

OTHER PUBLICATIONS

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CA 108: 26959 (1988).
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ART-UNIT: 152

PRIMARY-EXAMINER: Venkat; Jyothsna

ATTY-AGENT-FIRM: Foley & Lardner

ABSTRACT:

New biotenside esters, processes for their preparation and their use as solvents and and hydrotropic agents (coemulgators) in the preparation of spontaneously dispersible concentrates containing therapeutic or cosmetic agents are described.

16 Claims, 0 Drawing figures

L8: Entry 3 of 4 File: USPT Jan 14, 1997

DOCUMENT-IDENTIFIER: US 5593691 A
TITLE: Biotenside solvents for pharmaceuticals and cosmetics

CLAIMS:

0. 5 to 30% by weight of a therapeutic agent, a cosmetic agent, or a combination thereof,

up to 45% by weight of a phosphoric acid ester tenside or a tenside of the formula ##STR20## wherein M is hydrogen, an alkali or alkali earth atom and R.sub.x is a C.sub.1-32 alkyl, C.sub.2-32 alkenyl or a multifunctional glucose derivative selected from the group consisting of methyl glucose sesquistearate and PEG-20 methyl glucose sesquistearate, and

up to 45% by weight of a pharmaceutically acceptable tert.octyl-phenyl-polyoxyethylene ether having 9 or 10 oxyethylene groups.

3. A spontaneously dispersible concentrate as claimed in claim 2, wherein the phosphoric acid ester tenside is a mixture of

tristyrylphenolpolyoxyethylene-18-monomethyl-phosphoric acid ester, and tristyrylphenolpolyoxyethylene-18-di-phosphoric acid ester, a mixture of

nonylphenol-10-polyoxyethylene-monomethylphosphoric acid ester, and nonylphenol-10-polyoxyethylene-dimethylphosphoric acid ester, hydroxybiphenyl-10-ethoxy-phosphoric acid ester or butyl-mono-4-ethoxy-phosphoric acid ester and wherein the multifunctional glucose derivative is methyl glucose sesquistearate or PEG-20 methyl glucose sesquistearate.

4. A spontaneously dispersible concentrate as claimed in claim 1, wherein the concentrate comprises

20% by weight of a biotenside ester or a combination of biotenside esters according to claim 1, said concentrate further comprising:

10% by weight of an oily anti-tumor agent,

35% by weight of a pharmaceutically acceptable tert.octyl-phenyl-polyoxyethylene ether having 9 to 10 oxyethylene groups, and

35% by weight of a phosphoric acid ester tenside or a tenside of the formula ##STR21## wherein M is hydrogen, an alkali or alkali earth atom and R.sub.x is a C.sub.1-32 alkyl, C.sub.2-32 alkenyl or a multifunctional glucose derivative selected from the group consisting of methyl glucose sesquistearate and PEG-20 methyl glucose sesquistearate.

5. A spontaneously dispersible concentrate as claimed in claim 4, wherein the phosphoric acid ester tenside is a mixture of

tristyrylphenolpolyoxyethylene-18-monomethyl-phosphoric acid ester, and

tristyrylphenolpolyoxyethylene-18-dimethyl-phosphoric acid ester, a mixture of

nonylphenol-10-polyoxyethylene-monomethylphosphoric acid ester, and nonylphenol-10-polyoxyethylene-dimethylphosphoric acid ester, hydroxybiphenyl-10-ethoxy-phosphoric acid ester or butyl-mono-4-ethoxy-phosphoric acid ester and wherein the multifunctional glucose derivative is methyl glucose sesquistearate or PEG-20 methyl methyl glucose sesquistearate.

8. A pharmaceutical composition comprising 1 to 95% by weight of a spontaneously dispersible concentrate as claimed in claim 4 and up to 10% by weight of a pharmaceutically acceptable excipient, solvent or stabilizer, wherein the composition is formulated in unit dosage form as micropellets, granules, dragees, suppositories, ampules, or capsules.

10. A spontaneously dispersible concentrate as claimed in claim 9, wherein the concentrate comprises:

10 to 50% by weight of said biotenside ester or said combination of biotenside esters;

0.5 to 30% by weight of said therapeutic agent, said cosmetic agent, or said combination thereof;

up to 45% by weight of a phosphoric acid ester tenside or a tenside of the formula ##STR24## wherein M is hydrogen, an alkali or alkali earth atom, and R.sub.x is a C.sub.1-32 alkyl or a C.sub.2-32 alkenyl group, or a multifunctional glucose derivative selected from the group consisting of methyl glucose sesquistearate and PEG-20 methyl glucose sesquistearate; and said concentrate further comprising

up to 45% by weight of a pharmaceutically acceptable tert.octyl-phenyl-polyoxyethylene ether having 9 to 10 oxyethylene groups and/or a a fatty acid ester of polyoxyethylene sorbitan.

12. A spontaneously dispersible concentrate as claimed in claim 9, wherein the concentrate comprises:

20% by weight of said biotenside ester or said combination of biotenside esters;

10% by weight of an oily anti-tumor agent;

35% by weight of a pharmaceutically acceptable tert. octylphenylpolyoxy-ethylenether having 9 to 10 oxyethylene groups; and

35% by weight of a phosphoric acid ester tenside or a multi-functional glucose derivative selected from the group consisting of methyl glucose sesquistearate and PEG-20 methyl glucose sesquistearate.

16. A pharmaceutical composition comprising:

1 to 95% by weight of said spontaneously dispersible concentrate as claimed in claim 9; and

up to 10% by weight of a pharmaceutically acceptable excipient, solvent or stabilizer;

wherein said concentrate and said excipient, solvent or stabilizer are processed into dosage forms selected from the group consisting of micropellets, granules, coated tablets, tablets, and suppositories.